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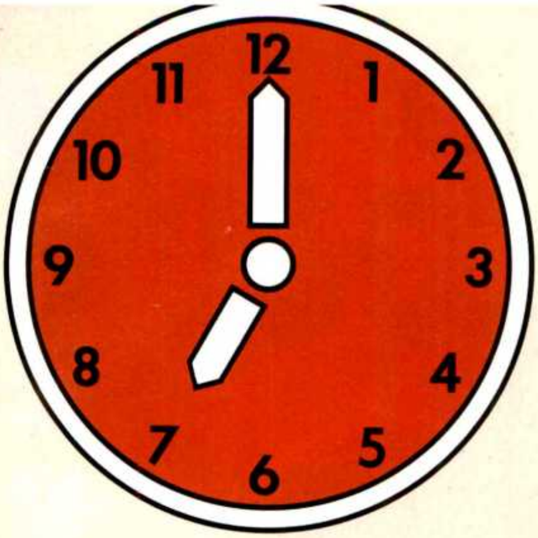

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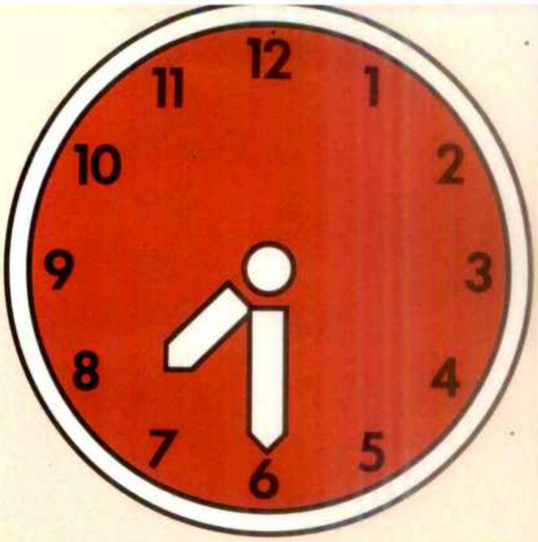

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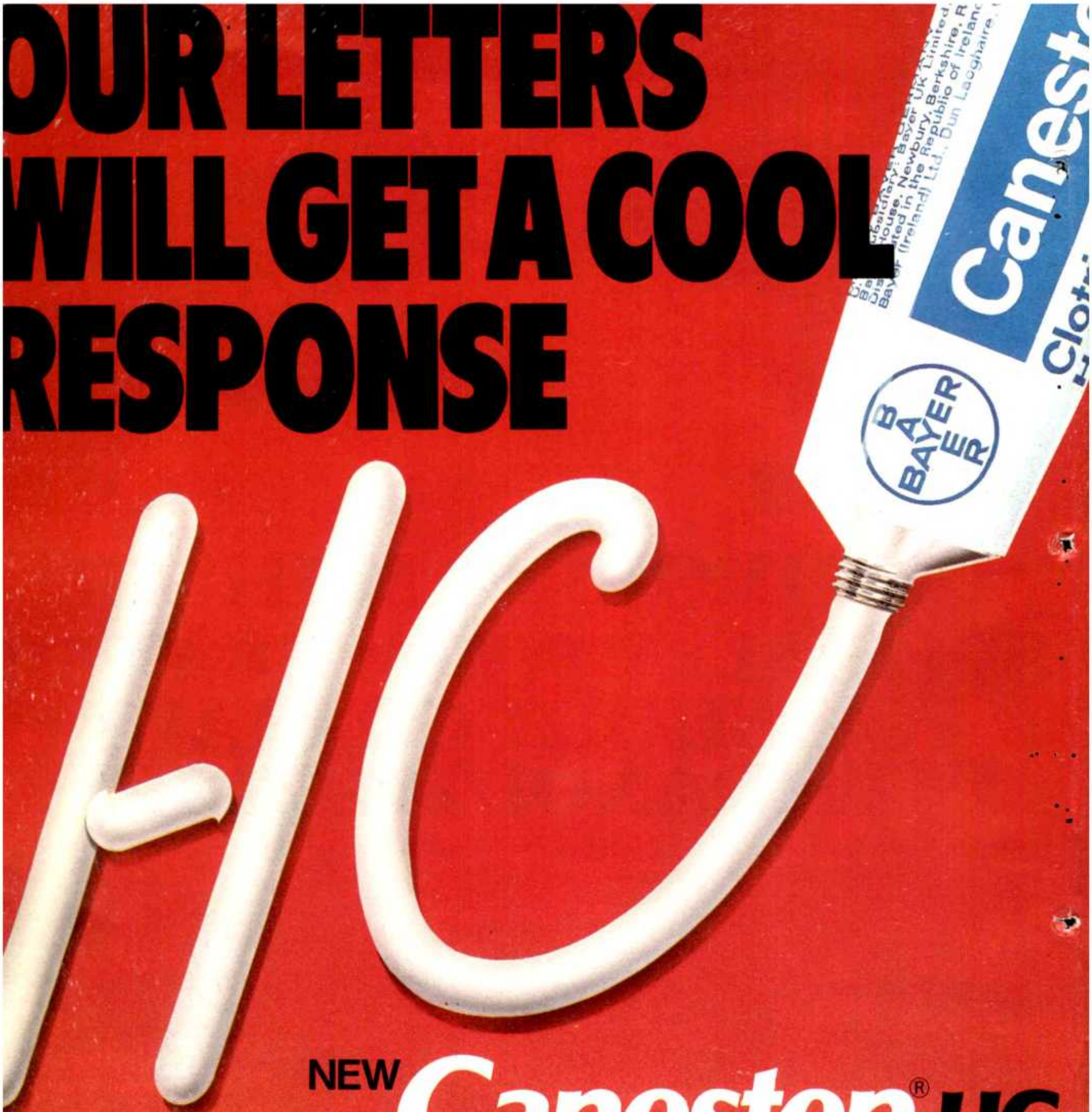

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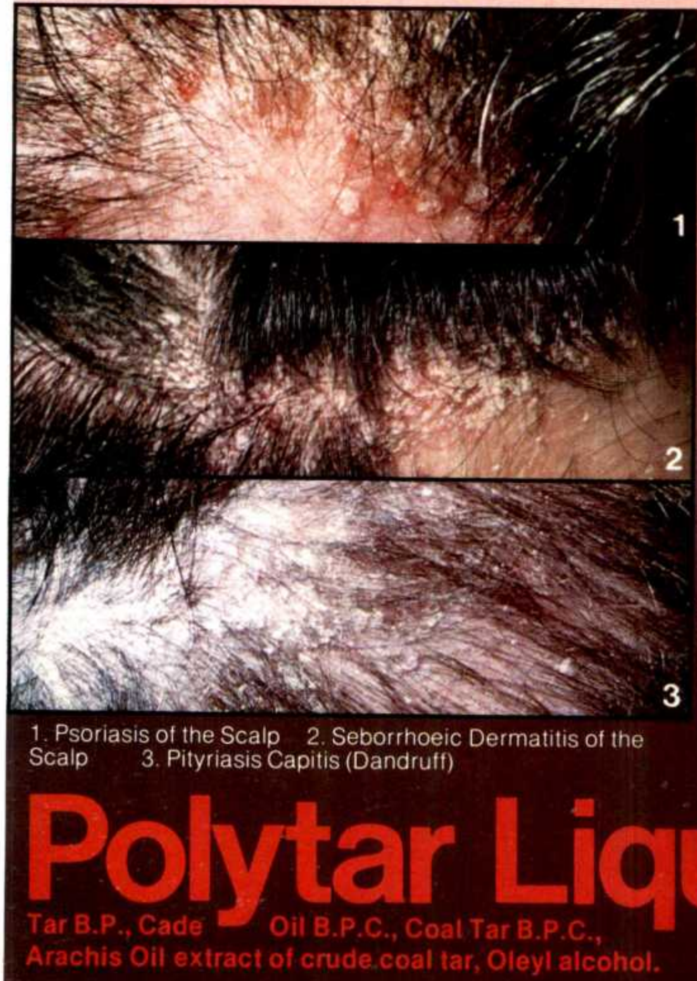

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Acknowledged as ideal for long-term control.

ALSO Polytar Plus with protein

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Presentation Polytar Liquid is a concentrated, antiseptic tar-medicated scalp cleanser containing the following active ingredients:

Tar B.P.	0.3%
Cade Oil B.P.C.	0.3%
Coat Tar B.P.C.	0.1%
Arachis Oil extract of crude coal tar	0.3%
Oleyl alcohol	1.00%

Polytar Plus contains 3.0% hydrolysed animal protein, in addition to the above.

Uses Polytar Liquid and Polytar Plus are indicated in the treatment of scalp disorders such as psoriasis, seborrhoea, dandruff, eczema and pruritus. They are also of value in the removal of pastes and pomades used in the treatment of psoriasis.

Dosage and Administration The hair should be wetted and sufficient applied to produce an abundant lather. The scalp and adjacent areas should be vigorously massaged with the fingertips. The hair should then be thoroughly rinsed and the procedure repeated.
Polytar Liquid or Polytar Plus should be used once or twice weekly.

Contra-indications, warnings, etc. Nil

Pharmaceutical Precautions Store in a cool place

Packs and Prices

	Pack	Basic NHS price
Polytar Liquid	65ml	91p
	150ml	£1.34
	350ml	£2.37
	1 litre	£6.18
Polytar Plus	150ml	£1.43
	350ml	£2.80

Product Licence Number
Polytar Liquid PL0174/5016 Polytar Plus PL0174/0037
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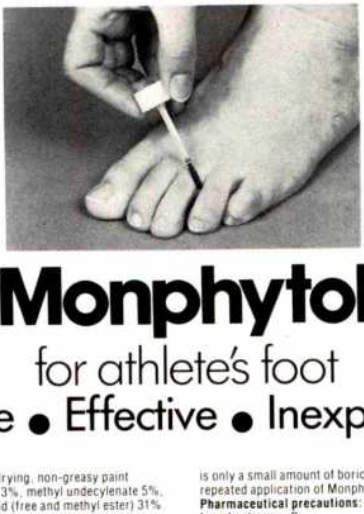

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Presentation: A colourless, rapidly drying, non-greasy paint containing boric acid 2%, chlorbutol 3%, methyl undecylenate 5%, propyl undecylenate 1%, salicylic acid (free and methyl ester) 31%.
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Legal category: P.
Package quantities: Bottles containing 18 ml with brush fitted in cap.
Further information: Nil.
Product licence number: 0118/5010.
Basic NHS price: £0.57 per 18 ml bottle.

Reference:
1. Krebs HA, Wiggins D, Stubbs M, Sols A, Bedoya F. Studies on the mechanism of the anti-fungal action of benzoate. Biochem J. 1983; **214**: 657-63.

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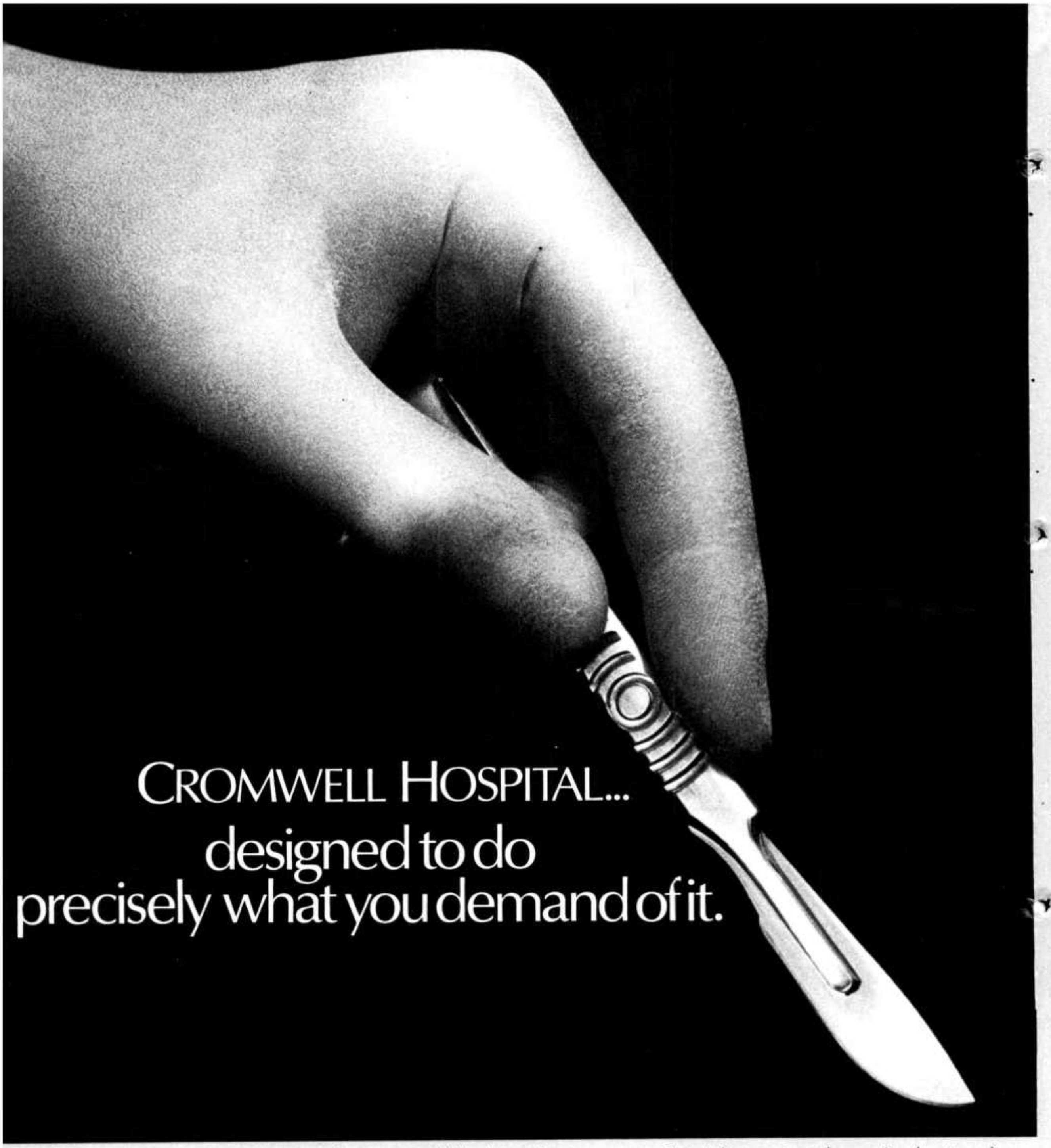
Lederle **Lederle Laboratories** Division of Cyanamid of Great Britain Ltd, Fareham Road, Gosport, Hants PO13 0AS (Tel: 0329-236131)



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T/GEL is a new medicated shampoo formulated as effective everyday therapy for scalp psoriasis, dandruff and seborrheic dermatitis. Regular use helps control itching, flaking and scaling while leaving the hair lustrous and manageable. T/GEL contains Neutar, a coal tar extract, equivalent in potency to 2% crude tar in suppressing D.N.A. synthesis (cell turnover). The unique clear T/GEL formula gives a rich lather, is pleasant to use, and free from lingering tar odour, encourages patient compliance.

PRESCRIBING INFORMATION

PRESENTATION T/GEL is a concentrated medicated tar based shampoo containing Neutar a solubilised coal tar extract 2% in a bland shampoo base consisting of: purified water, USP; sodium laureth sulfate; cocamide DEA; cocamidopopyl betaine; fragrance; imadazolidinyl urea; methylparaben; propylparaben; tetrasodium EDTA and citric acid.

USES Indicated as an effective aid in the treatment of psoriasis, dandruff and seborrheic dermatitis.

DOSAGE AND ADMINISTRATION Hair should be wetted thoroughly and a liberal amount of T/GEL applied and massaged into scalp, allow lather to remain on scalp for several minutes, then rinse, repeat application and rinse thoroughly.

PACK AND PRICE Pack 125 ml. Basic NHS price £1.52

PRODUCT & LICENCE NUMBER 5165/0001

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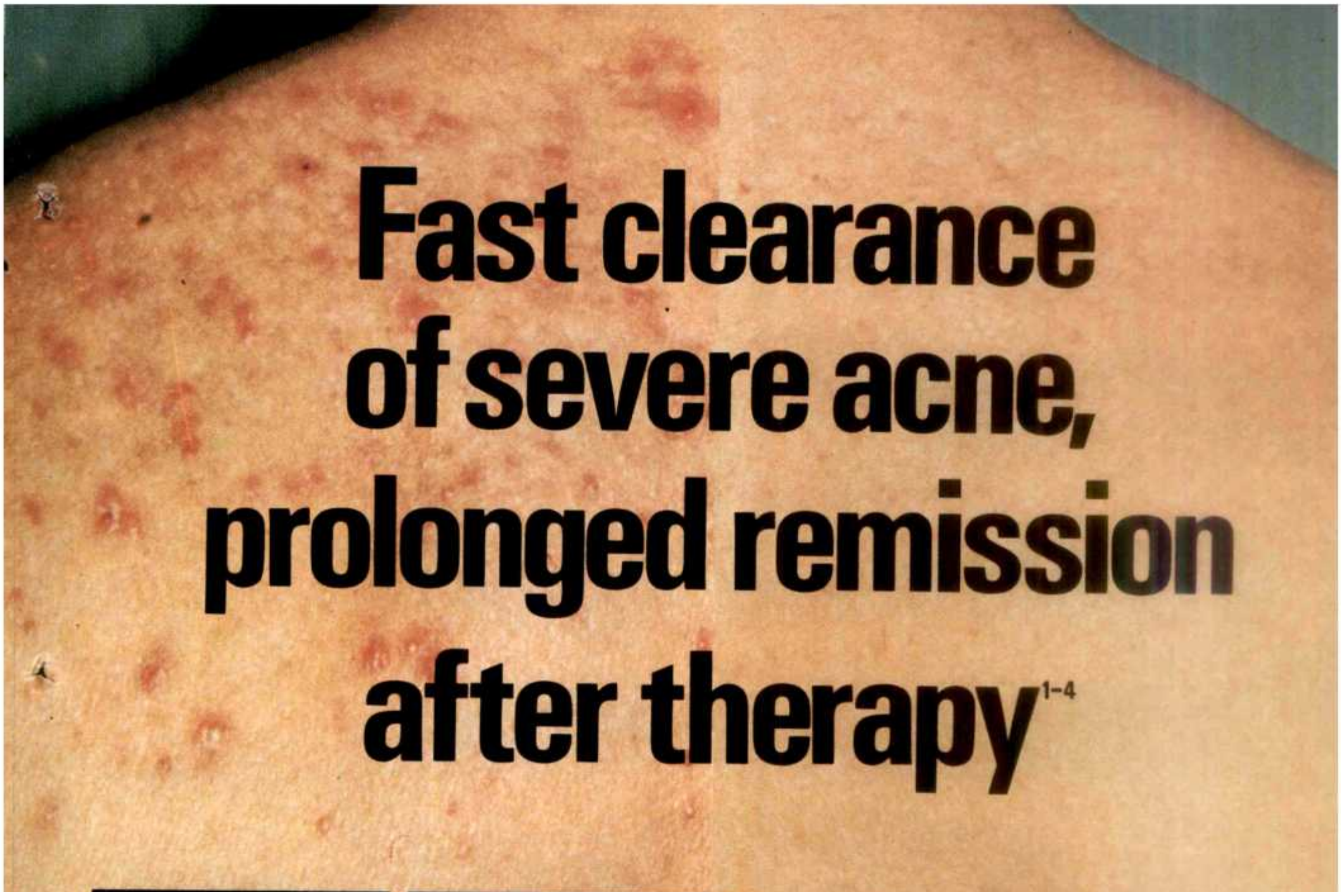
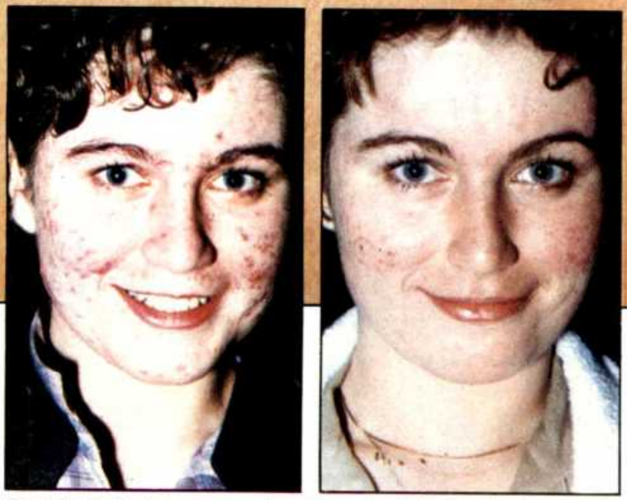
Application forms from: *Ministry of Defence, CM(S)4n, Lacon House, Theobalds Road, London WC1X 8RY. Tel: 01 430 6858/6363.*



Fast clearance of severe acne, prolonged remission after therapy[1-4]



Female patient unresponsive to oral antibiotics, before and after a course of Roaccutane.

"The aim of therapy is not only to produce clearing of active disease, but also to maintain this clearing indefinitely."[5]

In severe acne, Roaccutane rapidly produces an excellent response in 90% of patients and prolonged remissions after therapy once clearance is achieved.[1-4]

(Main picture) Male patient with truncal acne unresponsive oral antibiotics, before and after a course of Roaccutane.

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Contra-indications Pregnancy (Roaccutane is teratogenic—see 'Precautions') and breast-feeding. Hepatic or renal impairment.

Precautions The following precautions must be observed in women of childbearing potential.

1. Patients should be given detailed explanation of the risks.
2. Pregnancy should be excluded before therapy is instituted.
3. Effective contraception must be ensured for one month before treatment, during treatment, and for one month after treatment ends.

Use of patient information leaflets is strongly recommended. Monitor liver function and serum lipids (fasting value) regularly.

Side-effects Mainly mucosal dryness and fissures, facial dermatitis, desquamation, epidermal fragility, conjunctivitis; arthralgia/myalgia; increased hair loss (uncommon and reversible), possible elevation of liver enzymes and serum triglycerides. Benign intracranial hypertension has been reported, particularly in association with concomitant antibiotics.

Availability Hospitals and hospital clinics.

Presentations and Basic NHS Cost

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Product Licence Numbers 0031/0158 (capsules 5mg), 0031/0160 (capsules 20mg).

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References

1. In: "Retinoids: Advances in Basic Research and Therapy" Eds. Orfanos, C.E. *et al.* Springer-Verlag, Berlin, 1981, p.219
2. Brit. J. Derm., 1983, *108*, 333
3. J. Amer. Acad. Derm., 1982, 6, 735
4. J. Amer. Acad. Derm., 1982, 6, 766
5. J. Amer. Acad. Derm., 1983, 9, 629

Roaccutane is a trade mark.

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Contra-indications, Warnings, etc: Avoid contact with clothing and linen. Occasionally, transient stinging on application may occur. In cases of arterial or extensive, painful venous ulceration, this may be prolonged, when treatment should be discontinued until greater tolerance has been achieved. Keep away from the eyes.

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Green aqueous gel containing 0.5% w/w Brilliant Green BP, 0.5% w/w Lactic Acid BP.

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British Journal of Dermatology (1984) **111**, 1–11.

Clinical and Laboratory Investigations

Quantitative evaluation of two distinct cell populations expressing HLA-DR antigens in normal human epidermis

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Accepted for publication 24 January 1984

SUMMARY

HLA-DR antigens are expressed only by Langerhans cells (LC) and indeterminate cells (IC) in normal human epidermis. Indirect immunofluorescence and ultrastructural immunogold labelling were used to study the HLA-DR expression by means of two anti-DR monoclonal antibodies (MCA).

Freshly dispersed DR-positive epidermal cells expressed different densities of DR antigens on their membrane surface. Approximately 25% of DR-positive cells were strongly labelled by anti-DR-MCA and 75% were weakly stained. After 18 h in complete culture medium before labelling no significant difference in these percentages was observed. The lymphoid-like LC-enriched cells obtained by Ficoll-Hypaque centrifugation also had two populations of DR-positive cells: strongly labelled cells (30·8%) and weakly labelled cells (69·2%).

DR-positive cells may be divided into two types according to the density of DR sites on their cell membrane: (1) type DR^+ shows weak surface labelling by gold particles (8·8 ± 3·0 gold particles/μm) and has cytoplasmic Birbeck granules, identifying such cells as typical Langerhans cells; (2) type DR^{+++} shows strong membrane immunogold labelling (38·9 ± 4.6 gold particles/μm) and may or may not contain Birbeck granules. The gold particles bound to the cell membrane were used to quantify the number of HLA-DR sites expressed on each cell type: 1×10^5 sites on DR^+ cells and 5×10^5 on DR^{+++} cells.

Previous studies have shown that some epidermal cells (EC) express Ia (or HLA-DR) antigens (Hammerling *et al.*, 1975; Klareskog, Tjernlund & Forsum, 1977; Rowden, 1977a; Rowden, Lewis & Sullivan, 1977b; Stingl *et al.*, 1978). Two types of cells have been defined as HLA-DR positive cells in normal human epidermis: Langerhans cells (LC) with Birbeck granules and indeterminate cells (IC) without Birbeck granules (Rowden *et al.*, 1977b; Rowden, Phillips & Lewis, 1979). HLA-DR (Ia-like) antigens are considered to be highly specific markers for LC and their precursors in normal human epidermis. Most of the previous studies dealt with frozen skin sections and described the *in situ* expression of these cell surface antigens. In fact, our understanding of the expression and the function of these markers was extended by means of dispersed skin cells after trypsinization (Berle, Braathen & Thorsby, 1982; Hirschberg, Braathen & Thorsby, 1982). Preliminary experiments in our laboratory supported the evidence that cell surface HLA-DR antigen concentration distinguished two types of cells in the human epidermis, as shown by immunoperoxidase and electron microscopic observations performed on human EC suspensions (Czernielewski *et al.*, 1983). Typical LC with Birbeck granules exhibited a specific staining limited to the plasma membrane (DR$^+$ cells) and Birbeck granule lacking cells, called indeterminate cells, exhibited a more intense staining (DR^{+++} cells) (Czernielewski *et al.*, 1983). Therefore it seemed worth characterizing more precisely these two types of DR-positive cells.

In the present study, we approached this characterization by means of two immunolabelling procedures: (1) immunofluorescence (IF) techniques for the estimation of relative proportion of cells expressing both types of HLA-DR density; fluorescence microscopy in crude epidermal cell populations and cytofluorimetry in Ficoll-Hypaque enriched EC populations; (2) an ultrastructural immunogold analysis (Schmitt *et al.*, 1983) performed on EC suspensions enriched for DR-positive EC by a panning method. This immunogold labelling demonstrated the existence of two different densities of DR sites on the cell membranes, which correlated with the type of DR-positive cells.

METHODS

Preparation of epidermal cell suspensions

Single-cell suspensions were prepared from freshly removed normal human skin (plastic surgery, breast and abdomen). Briefly, trimmed skin was split-cut with a keratotome set at 0·1 mm. The resulting slices were treated for 60 min at 37°C with 0·3% trypsin (Difco, Detroit, U.S.A.) in buffer (0·68% NaCl, 0·04% KCl, 0·1% glucose, 0·22% $NaHCO_3$, pH 7·3). The epidermis was detached from the dermis with fine forceps. Isolated epidermal samples were pooled in Minimum Essential Medium (MEM, Gibco, Glasgow, U.K.) supplemented with 20% fetal calf serum (FCS) and repeatedly blown in and out of a 5 ml pipette. After filtration through sterile gauze, the suspended cells were centrifuged for 20 min at 400 ***g***. The collected cells were resuspended in RPMI 1640 (Gibco, Glasgow, U.K.) supplemented with 15% heat-inactivated AB human serum, 20 mmol/l Hepes buffer, 2 mmol/l L-glutamine and antibiotics. Following a triple wash in the RPMI medium, the cells were counted and the viability estimated by Trypan Blue exclusion. Viability, after trypsinization, was 85% or higher and after 18 h maintenance exceeded 75%.

Enrichment of epidermis cell suspensions

For immunoelectron microscopic studies, DR-positive epidermal cells were enriched using a modification of the plate technique described by Wysocki and Sato (1978) and Reinherz *et al.* (1981). Briefly, plastic plates (Corning, NY, U.S.A.) were coated with affinity-purified goat anti-mouse Ig (Tago Inc., Burlingame, U.S.A.). Concurrently, isolated EC were incubated with BL_2 MCA (diluted 1:200), for 1 h at room temperature, then washed twice in PBS. Three ml of the cell suspensions at a concentration of 5–6 × 10^6/ml was applied to a single goat anti-mouse Ig-coated plate. After 60 min the supernatant was removed. The adherent cells were then removed by repeated scraping with a rubber policeman and processed for immunogold labelling.

For cytofluorimetric analysis and cell sorting, enrichment was obtained by Ficoll-Hypaque (Pharmacia France SA, Le Chesnay, France) sedimentation. Dispersed skin cells (4×10^6 cells/ml) in TC 199 (Gibco, Glasgow, U.K.) supplemented with 20% FCS were layered on the gradient and centrifuged for 30 min at 400 ***g*** at 4°C. The lymphoid-like cells at the interface and the cells in the pellet were washed and resuspended in phosphate-buffered saline (PBS) pH 7·2, containing 30 mM EDTA and 1% bovine serum albumin (BSA). EDTA was added to the PBS to decrease cell clumping, and BSA to prevent non-specific absorption.

Monoclonal antibodies

Two anti-HLA-DR monoclonal antibodies were used.

(1) BL_2 is a MCA produced by J.Brochier, the specificity of which was shown to be identical to that of monomorphic anti-HLA-DR. It belongs to the IgG_{2b} isotype and was produced by mouse hybridoma (Cohen *et al.*, 1981; Czernielewski *et al.*, 1982; Yonish-Rouach *et al.*, 1982).

(2) MAS 054 is a MCA obtained commercially (Sera-Lab). This MCA is secreted by hybridomas formed by the fusion of a Y3 Ag-1-2-3 rat myeloma cell with spleen cells from a rat immunized against Daudi cells. It reacts with a monomorphic determinant on the HLA-DR molecule on human cells.

Immunofluorescence staining

Fluorescence microscopy. The binding of monoclonal antibodies to epidermal cells was studied by indirect IF. Trypsinized skin cells were washed twice with RPMI 1640 supplemented with 5% FCS, 20 mmol/l Hepes buffer, and stained by suspending 5×10^5 cells in 0·1 ml MCA for 1 h at 20°C; BL_2 MCA and MAS 054 MCA were applied at a dilution of 1:800. After two washes with RPMI medium, the cells were further incubated for 30 min at 20°C with fluorescein isothiocyanate-conjugated goat anti-mouse IgG (G/M-FITC, Tago, Inc., Burlingame, Calif., U.S.A.) or fluorescein isothiocyanate-conjugated rabbit anti-rat IgG (R/R-FITC, Zymed laboratories, Burlingame, Calif., U.S.A.). G/M-FITC and R/R-FITC were applied at a dilution of 1:40. Following two subsequent washes, the cells were examined with a Zeiss fluorescence microscope. A minimum of 1×10^3 cells were counted. Control cells were incubated with normal mouse and rat sera diluted 1:200 in the first IF step.

Cytofluorimetry. The lymphoid-like cells and the pelleted cells suspended in PBS, 30 mM EDTA, 1% BSA, were subjected to immunofluorescence labelling as outlined above. Cytofluorimetric analysis and cell sorting were performed only on a portion of the lymphoid-like cells fixed with paraformaldehyde in PBS (1%, v/v) as explained in the Results. The samples were run in a cytofluorograf 50 H (Ortho instruments, Wertwood, Ma.) interfaced with an interactive computer developed locally (Cordier, Lefebvre & Revillard, 1983) which allows simultaneous analysis of scattered light and fluorescence signals for each cell passing through the laser beam. Forward angle light scatter (FAS) and right-angle light scatter (RAS) allowed the analysis of cells according to their diameter and their content; the intensity of the fluorescence signal is related to the number of fluorescein molecules present on the surface of the cell. Evaluation of percentages of labelled cells were done by setting a threshold on the histogram by comparison with background fluorescence determined on cells treated with normal mouse serum. Determinations were based on 1×10^4 cells per sample. Fluorescence intensity and cell type analysis were checked on sorted cells according to their light scattering properties.

Immunogold labelling procedures and quantitative analysis

EC suspensions were fixed by 2% paraformaldehyde in periodate–lysine buffer. After washing, BL_2 MCA was applied to specimen cells for 30 min at 37°C at a dilution of 1:10. The murine antibodies, fixed on the cell surface, were identified by the use of goat anti-mouse immunoglobulin labelled with gold granules of 20 nm size (GAM 20, Janssen, Pharmaceutica, Beerse, Belgium) applied at a dilution of 1:2 for 1 h at 37°C. After washing with PBS, the cells were post-fixed for 20 min with 2% glutaraldehyde in cacodylate buffer, then for 20 min with 1% osmium tetroxide and embedded in epoxy medium. Ultrathin sections were examined after post-staining with lead citrate and uranyl acetate with an Philips EM 300 electron microscope (Centre de Microscopie Electronique, CMEABG, Université Lyon I, Villeurbanne).

The number of gold particles along the circumference of the cell membrane was counted to determine the extent of surface labelling. Mean and standard errors for each morphological group were determined. The cells analysed were those sectioned near their midplane, to minimize tangential membrane sections and to provide a uniform morphological comparison between cells. Labelling data are reported as the mean number of gold particles per micrometre of membrane (gp/μm). Particle counts were obtained from at least ten cells in each population.

The number of gold particles per μm was used to estimate the approximate number of cell surface antigenic sites (Trucco & De Petris, 1981; Trucco *et al.*, 1980). Diameters of at least forty DR-positive cells were measured in different subjects and the mean diameter of the studied cells was calculated and found to be 15 μm.

RESULTS

Fluorescence microscopic analysis of HLA-DR antigens on freshly isolated and short-term cultured epidermal cells

Dispersed crude human epidermal cells labelled with either BL_2 MCA or MAS 054 MCA revealed specific staining of approximately 2±0·6% (standard deviation, s.d.) of the total

TABLE 1. Quantitation of HLA-DR antigen expression by means of fluorescence microscopy and cytofluorimetry in crude epidermal cell suspensions and lymphoid-like Langerhans cell-enriched epidermal cell suspensions

		Fluorescence microscopic analysis.		
		Crude epidermal cell suspensions		Cytofluorometric analysis. Lymphoid-like LC-enriched epidermal cell suspensions
Monoclonal antibody	Nature of %	Freshly dispersed cells	Short-term-cultured cells	
BL_2 or MAS 054	$\frac{\text{Labelled EC}}{\text{Total EC}} \times 100$	mean 2·0% (s.d. 0·6%) $n = 14$	mean 2·8% (s.d. 0·8%) $n = 12$	mean 11·8% (s.d. 5·8%) $n = 10$
BL_2	$\frac{\text{Intensely labelled EC}}{\text{Total labelled EC}} \times 100$	mean 24·5% (s.d. 4·5%) $n = 8$	mean 27·5% (s.d. 4·9%) $n = 7$	mean 30·8% (s.d. 11·4%) $n = 9$
MAS 054	$\frac{\text{Intensely labelled EC}}{\text{Total labelled EC}} \times 100$	mean 21·8% (s.d. 4·6%) $n = 6$	mean 22·6% (s.d. 4·8%) $n = 5$	ND

s.d. = standard deviation.
n = number of samples.
ND = not done.

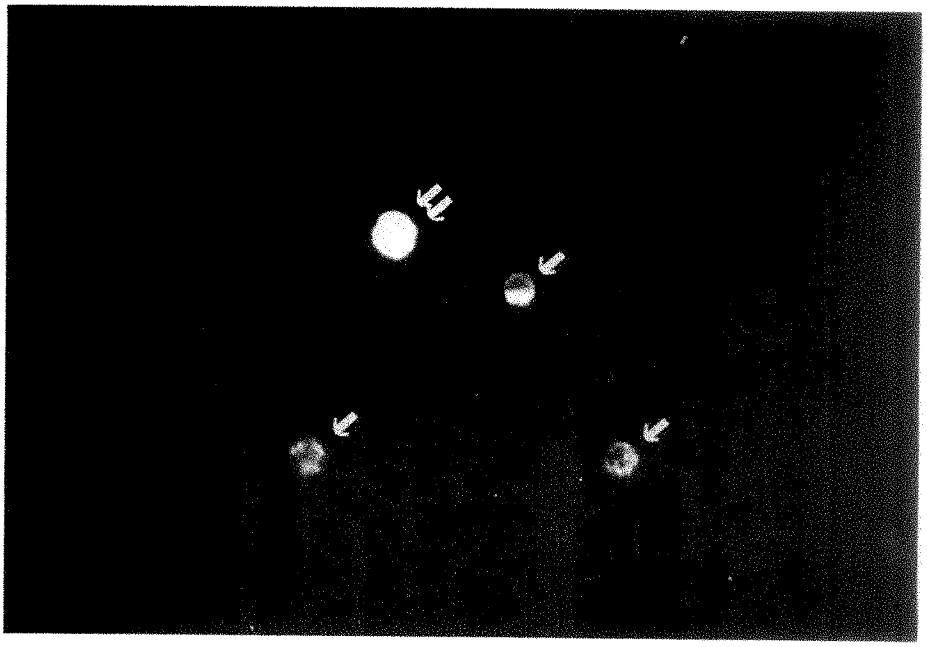

FIGURE 1. Detection of HLA-DR antigens on dispersed skin cells. Human skin was trypsinized, resuspended in RPMI 1640 medium and stained with anti-DR monoclonal antibodies (BL_2 MCA plus G/M FITC or MAS 054 MCA plus R/R/FITC). Strongly DR-positive cells (⇒); weakly DR-positive cells (→).

normal EC (Table 1). Unlabelled skin cells incubated with normal mouse serum or normal rat serum plus the appropriate fluoresceinated immunoglobulin showed no fluorescence. Two intensities of staining were observed: (a) some strongly positive cells having regular, dense lineo-granular fluorescent deposits on the cell membrane and (b) some weakly and moderately positive cells with irregular, speckled fluorescent deposits (Fig. 1). The percentage of intensely BL_2 MCA-labelled cells was similar to that obtained with MAS 054 MCA (Table 1). Although a similar number of strongly DR-positive cells was found after 18 h culture (Table 1) we observed that their labelling was somewhat brighter than in freshly isolated epidermal cells.

Cytofluorimetric analysis of HLA-DR antigens on lymphoid-like epidermal cells

Skin cell suspensions were centrifuged through a Ficoll-Hypaque gradient. The pellet and interface were collected and labelled with anti-DR-MCA (BL_2) which revealed specific staining of approximately $0{\cdot}5 \pm 0{\cdot}6\%$ of pelleted cells and $17{\cdot}0 \pm 6{\cdot}1\%$ of lymphoid-like cells.

Enrichment of LC by Ficoll-Hypaque sedimentation did not select any type of DR-positive cells since the percentages of intensely BL_2 MCA-labelled cells were not significantly different in crude EC suspensions ($24{\cdot}5 \pm 4{\cdot}5\%$, $n = 14$) (Table 1), in lymphoid-like cell populations ($24{\cdot}8 \pm 5{\cdot}4\%$, $n = 9$) and in non-lymphoid-like cell populations ($23{\cdot}7 \pm 5{\cdot}7\%$, $n = 9$).

Following Ficoll-Hypaque sedimentation of EC, population analysis with the cytofluorimeter showed the slight enrichment of BL_2-labelled cells (Table 1). With a scatter setting eliminating very large cells and cell clumps, fluorescence profiles of normal mouse serum-treated EC (Fig. 2A) and BL_2-treated EC (Fig. 2B) were compared. The first peak was the

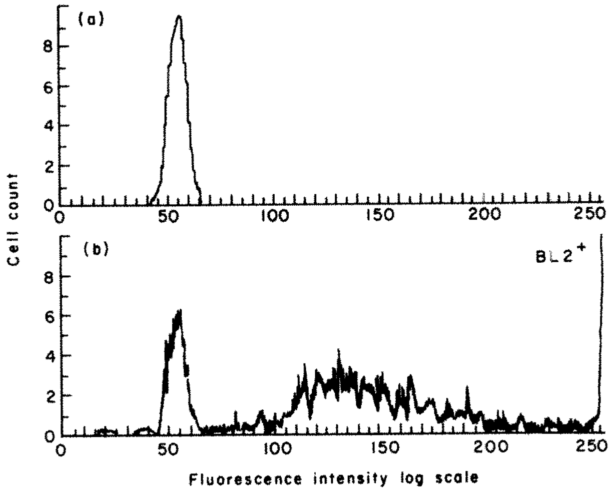

FIGURE 2. Fluorescence profiles of lymphoid-like epidermal cell suspensions labelled with normal mouse serum (a) or anti-DR (BL_2 MCA) antibodies (b) plus the appropriate FITC-Ig. Cells were prepared and labelled as described in Methods. Analysis of 1 × 10^4 cells of each type was performed.

negative fraction of cells; the DR-positive fraction of cells was represented by the other peaks included in the fluorescence window of > 70. Using a threshold set at the fluorescence window of > 210 we observed brightly DR-positive cells accounting for 30·8 ± 11·4% of positive EC (Fig. 2 and Table 1). Cells were sorted according to their fluorescence or non-fluorescence. For fluorescent cells, populations of weakly and strongly labelled cells were again observed (Fig. 1).

Ultrastructural observation of surface labelling of DR-positive cells

HLA-DR labelled cells could be identified by the presence of variable numbers of electron-dense gold particles scattered along the cell surface. The labelling appeared as single particles, small clusters or occasionally as small arrays of particles in direct apposition with the plasma membrane. No gold particles were observed within the cytoplasm of these cells. Keratinocytes and melanocytes were negative.

Two distinct types of DR-positive cells could be identified according to the number of gold particles bound to the cell membrane. The majority of cells showed a weak labelling with a relatively low number of gold particles along the membrane. Those cells were always noted to contain Birbeck granules and were considered as typical Langerhans cells (Fig. 3). Some cells showed a strong labelling characterized by a larger number of gold particles regularly scattered along the cell membrane. These last cells included typical LC. In addition, some cells lacking Birbeck granules, with no features of keratinocytes or melanocytes, exhibited this same intense labelling. They could be defined as indeterminate cells, with cytoplasmic vesicles, dense mitochondria and numerous filaments.

Quantitative estimation of DR sites on epidermal cells

The estimations of the mean surface labelling density with standard errors for DR-positive cells are shown in Table 2. The weakly and moderately labelled cells had a mean particle-binding density ranging from 3·6 to 11·2 gp/μm of cell circumference (mean 8·8 ± 3·0 gp/μm, n = 10).

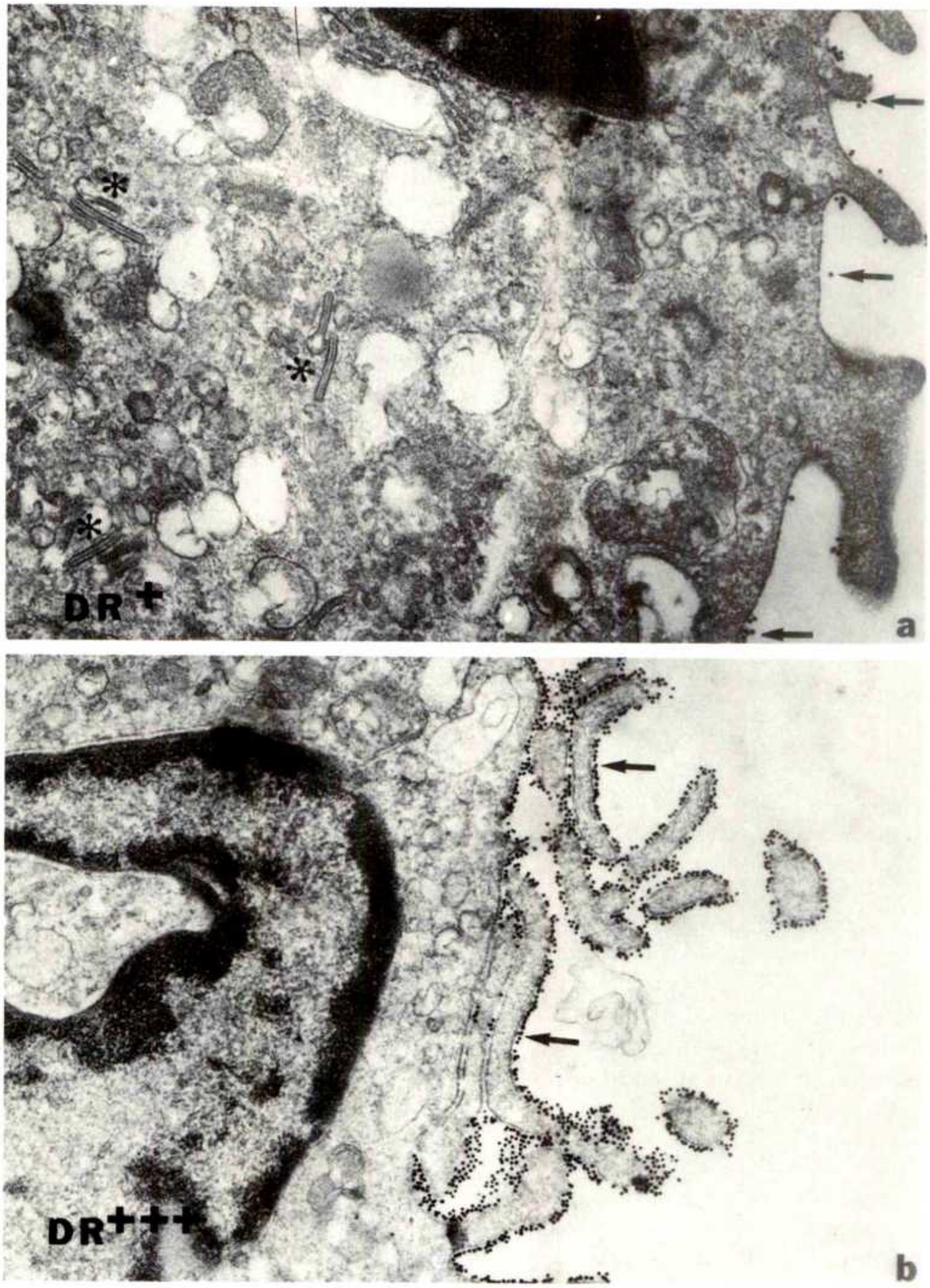

FIGURE 3. High magnification of DR-positive EC. (a) A DR^{+} cell with a weak labelling and a low number of gold particles along the membrane (→), typical Langerhans cell with Birbeck granules (*). (b) A DR^{+++} cell lacking Birbeck granules and showing a strong labelling with a large number of gold particles (original magnification × 54,000).

TABLE 2. Quantitation of HLA-DR antigen expression in epidermal cell suspensions

HLA-DR positive cells	DR$^+$ cells	DR^{+++} cells
Number of gold particles/μm[a]	8·8 ± 3·0	38·9 ± 4·6
Estimated number of HLA-DR sites/cell[b]	1×10^5	5×10^5
% Each type of cell (light microscopy)[c]	75·5	24·5
% Each type of cell (cytofluorometry)[d]	69·5	30·8

[a] Mean gold particle counts (± s.d.) per μm of cell membrane from ten cells of each type. Ultrastructural immunogold labelling of cell suspensions enriched by panning.

[b] Based on the method of Trucco *et al.* (1980).

[c] Percentage of DR$^+$ and DR^{+++} cell populations among DR-positive cells from crude EC suspensions determined by IF analysis using BL$_2$ MCA.

[d] Percentages of DR$^+$ and DR^{+++} cell populations among DR-positive EC obtained by cell sorting analysis on Ficoll-Hypaque gradient-enriched epidermal cells.

The density on intensely labelled cells ranged from 31·4 to 43·1 gp/μm of cell circumference (mean 38·9 ± 4·6 gp/μm, $n = 10$).

To estimate the number of DR sites labelled on each group of cells the following assumptions were made: one molecule of MCA reacts with one antigenic DR site and one molecule of gold-conjugated immunoglobulin reacts with only one molecule of MCA. No enhancing effect is to be expected because the second antibody is fixed on a granular solid phase which is presently the gold particles of 20 nm (Trucco *et al.*, 1980; Trucco & De Petris, 1981). The number of sites estimated by the method of Trucco and De Petris (1981) is approximately 1×10^5 DR sites on intensely labelled cells. In this calculation both types of cells were considered as spheres of 15 μm in diameter.

DISCUSSION

DR antigens can be detected on the surface of some human EC by immunoflorescence, immunoperoxidase or immunoelectron microscopy (Hammerling *et al.*, 1975; Klareskog *et al.*, 1977; Morhenn *et al.*, 1982; Rowden, 1977a; Stingl *et al.*, 1978). Our present data clearly indicate that by IF of normal human EC suspensions two different intensities of immunolabelling can be noted on DR-positive cells. Approximately 25% of the positive cells are strongly labelled while the rest of the DR-positive cells are moderately or weakly labelled, as shown by both fluorescence microscopy and cytofluorimetric analysis. This confirms and extends our initial observation by immunoelectron microscopy on suspensions of normal human EC, where two labelling patterns were defined on the basis of the intensity of peroxidase products on cell membrane (Czernielewski *et al.*, 1983). This is unlikely to be due to a loss of membrane markers during the trypsinization procedure, since a similar difference persisted even after a short-term culture of isolated EC. It thus ruled out the possibility of artifact due to the effect of prior trypsinization, as suggested by Morhenn *et al.* (1982).

Among the DR-positive cell population, the distribution of intensely and moderately labelled cells did not seem to be affected by the method of enrichment. The DR-positive cells were not homogeneous in fluorescence intensity and in membrane HLA-DR density. As shown in crude EC suspensions, some of these enriched positive cells had a high density of HLA-DR antigens as compared with the majority of the moderately and weakly positive cells.

The ultrastructural immunogold labelling was used for a better identification of EC expressing the HLA-DR antigens among normal human EC, and to enable the estimation of antigen densities on HLA-DR-positive EC. Because of the low percentage of DR-positive cells among EC, enrichment of DR-positive cells was necessary. The panning method as previously reported by Reinherz *et al.* (1981), Scheynius *et al.*, (1982), Wysocki and Sato (1978) and ourselves (Czernielewski *et al.*, 1983) was used, although it gave only moderate enrichment. Since its first report (Page-Faulk & Taylor, 1971), the immunogold labelling method has been largely used for the identification of cell surface antigens on cell suspensions by immunoelectron microscopy (De Mey, 1983; Horisberger, 1979; Lafferty *et al.*, 1981) including quantitative evaluations of labelling densities of various cell surface markers (Lafferty *et al.*, 1981). Various advantages of this method can be noted: the absence of non-specific absorption of gold particles on cell surfaces, the possibility of post-staining ultrathin sections without changes in the immunolabelling contrast and of a double labelling using gold particles of different sizes. As demonstrated by Horisberger (1979) and Lafferty *et al.* (1981), the number of DR sites may be estimated from the densities of gold particles bound to the surface, keeping in mind that steric hindrance resulting from the size of the label allows exclusion of an amplification phenomenon (Lafferty *et al.*, 1981). According to this estimation, we calculated the number of DR-sites/cell and we found a majority of cells with 1×10^5 DR-sites/cell and a few cells with 5×10^5 DR-sites/cells. These results are consistent with those of Trucco *et al.* (1980) on a B-cell line ($4{\cdot}6 \times 10^5$ sites/cell), and on B cells among peripheral blood lymphocytes ($0{\cdot}8 \times 10^5$ sites/cell). The use of this technique allowed us to quantify the differences previously observed by immunoperoxidase technique (Czernielewski *et al.*, 1983).

Langerhans cells, as defined by the presence of intracytoplasmic Birbeck granules, displayed different densities of HLA-DR antigens by immunoelectron microscopy. Among intensely DR-positive cells, some cells could be identified as indeterminate cells because they lacked intracytoplasmic Birbeck granules. It is unlikely that they represent Langerhans cells, the granules of which were out of the plane of the section, since no EC lacking Birbeck granules displayed a low density of HLA-DR antigens. Perhaps the indeterminate cells with a high density of HLA-DR antigens may represent activated and immature states of LC or functionally distinct subtypes. The T_6 antigen is now considered as a highly specific differentiation marker of LC (Chu *et al.*, 1982; Harrist *et al.*, 1983; Holden, Morgan & MacDonald, 1982; Murphy *et al.*, 1982, 1983). Monoclonal anti-T_6 antibody also reacts with dermal indeterminate cells (Murphy *et al.*, 1983). The exact relationships between Langerhans cells, the so-called indeterminate cells of the epidermis, and the EC expressing different densities of HLA-DR would certainly benefit from double-labelling studies using anti-T_6 and anti-DR antibodies, both in IF and immunoelectron microscopy. Moreover, the fact that two EC subpopulations can be distinguished in terms of membrane DR antigen densities may suggest a difference in the capacity of antigen presentation between these two types of cells. Further studies will be necessary to define the functional ability of these different DR-positive cells *in vitro* using the mixed skin cell lymphocyte reaction method and, as stimulating cells, EC suspensions enriched for each cell type.

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In situ immunological characterization of the infiltrating cells in positive patch tests

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SUMMARY

Skin biopsies from positive allergic patch tests were analysed by immunoenzymatic labelling of frozen sections with monoclonal antibodies. In seventeen patients the cellular infiltrate consisted of T cells admixed with Langerhans cells/indeterminate cells, but in two patients there were also many B lymphocytes. The B cells were accompanied by dendritic reticulum cells forming B-cell follicles, indistinguishable from those of normal and hyperplastic lymph nodes. There was no correlation between these two immunohistological staining patterns and the sensitizing antigen, the extent of local reaction or the time from epicutaneous application of allergen to examination (2 to 16 days). The ratio between T-helper and T-suppressor cells varied considerably, and showed no correlation with these variables. In all patients the infiltrating T cells expressed HLA-DR antigen. Transferrin receptors were identified on the infiltrating T cells in biopsies from nine patients.

These data indicate activation of T cells in the infiltrate from positive patch tests, and support the functional significance of Langerhans cells in the initiation and maintenance of cutaneous contact allergy. An involvement of B cells and B-cell accessory cells in the pathogenesis of contact allergic reactions is also suggested. The presence of dendritic reticulum cells in skin infiltrates from positive patch tests may reflect a functional implication of the skin in the development of B-cell memory.

A positive patch test is considered to result from a delayed type hypersensitivity of the skin. The importance of Langerhans cells in the initiation and maintenance of cutaneous contact allergy is well established (Toews *et al.*, 1980), but limited information is available concerning the participation of other types of lymphoid cells in the reaction.

The purpose of this study was to determine the cell types in skin infiltrates from positive patch tests by immunoenzymatic labelling of lymphocytes and accessory cells in frozen sections with monoclonal antibodies.

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METHODS

Tissue specimens

Four mm skin punch biopsies were obtained from nineteen patients with positive patch tests 2–16 days after exposure to different allergens (Table 1). The allergens were applied on to the skin of the upper back, left occluded for 24 h, and read at 48 h. A positive reaction was scored + to + + + + according to the degree of erythema, infiltration and vesiculation. Skin punch biopsies from six adult, healthy volunteers were also analysed.

Preparation of frozen sections

Biopsies were frozen and stored in liquid nitrogen. Six-micron cryostat sections were lyophilized at −20°C for 2–4 h, fixed in acetone for 10 min, and either washed and stained immediately after fixation or stored at −20°C for 2–4 weeks before staining. In the latter instance sections were fixed in acetone for an additional 10 min before staining (Stein *et al.*, 1982).

Monoclonal antibodies

The specificities and sources of the monoclonal antibodies are detailed in Table 2. 3AI was kindly made available by Dr B.F.Haynes, National Institute of Allergy and Infectious Disease, Bethesda, Maryland.

Immunoenzymatic staining

Sections were incubated with anti-human monoclonal antibodies (30 min), washed in

TABLE 1. Clinical data from nineteen patients with positive patch tests

Patient	Age	Sex	Allergen	Local reaction	Days from application of allergen to biopsy
1	51	M	Nickel	+ +	2
2	23	M	Benzoin	+ + +	2
3	52	M	Chromium	+ + +	2
4	33	F	Nickel	+ + +	2
5	64	M	Colophony	+ + + +	2
6	28	M	Paraphenylenediamine	+ +	2
7	50	M	Fragrance-mix	+ +	2
8	50	M	Chromium	+ + +	3
9	36	M	Cobalt	+ + +	3
10	51	M	Cobalt	+ + +	3
11	66	F	Neomycin	+ + +	4
12	48	M	Thiuram mix	+ +	5
13	56	M	PPD mix	+ + +	5
14	58	M	Neomycin	+ + + +	5
15	48	F	Chloramphenicol	+ + +	5
16	71	F	Thiuram mix	+ +	5
17	72	F	Neomycin	+ +	5
18	65	F	PPD mix	+ +	6
19	63	F	Balsam of Peru	+ +	16

TABLE 2. Anti-human monoclonal antibodies

Antibody	Specificity	References	Commercial Source*
Leu1/Lyt2	Pan T cells	Martin *et al.* (1981)	BD/NEN
Leu5/Lyt3	Pan T cells (E-rosette receptor)	Kamoun *et al.* (1981)	BD/NEN
Leu4	Peripheral T cells	Reinherz & Schlossman (1980)	BD
Leu3	T-helper/inducer cells	Ledbetter *et al.* (1981)	BD
Leu2	T-suppressor/cytotoxic cells	Ledbetter *et al.* (1981)	BD
3A1	T-helper/inducer cells. Concanavalin inducible T-suppressor cells	Haynes (1981)	—
OKT6	Cortical thymocytes, Langerhans cells	Reinherz & Schlossman (1980) Fithian *et al.* (1981)	Ortho
OKT9	Transferrin receptor	Terhorst *et al.* (1981)	Ortho
OKT10	Precursor cells, activated B and T cells	Terhorst *et al.* (1981)	Ortho
Leu7	Natural killer cells	Abo & Balch (1981)	BD
B1	Pan B cells	Stashenko *et al.* (1980)	CC
To15	Pan B cells	Stein *et al.* (1982)	DAKO
R4/23	Dendritic reticulum cells	Naiem *et al.* (1983)	DAKO
To5	C3b-receptor	Gerdes *et al.* (1982a)	DAKO
MO2	Monocytes	Todd *et al.* (1981)	CC
CR3/43	HLA-DR antigen	Gatter *et al.* (1981)	DAKO

* Becton-Dickinson (BD); New England Nuclear (NEN); Ortho Diagnostics (Ortho); Coulter Clone (CC); Dakopatts (DAKO).

Tris-buffered saline, and labelled with peroxidase-conjugated rabbit anti-mouse immunoglobulin (Dakopatts) diluted 1 : 10 with phosphate-buffered saline with 33% (v/v) normal human serum (30 min). Sections were thereafter washed, stained with 3-ethyl-9-aminocarbazol and H_2O_2, counter-stained with Mayer's haematoxylin, and mounted in Aquamount®.

For each antibody the number of positive cells was estimated by counting labelled cells relative to the total number of nuclei within the fields of a 5 × 5 mm ocular net at × 400 magnification. The number of OKT6-positive epidermal Langerhans cells was estimated as previously detailed (Harrist *et al.*, 1983). Thus only cells demonstrating dendrites in association with nuclei were scored as positive (Fig. 1).

Positive controls were performed by staining frozen sections of human hyperplastic tonsils or lymph nodes. Negative controls were performed by omitting the specific anti-human monoclonal antibodies.

RESULTS

Immunohistological examination of frozen sections of biopsies from positive patch tests with a panel of monoclonal antibodies demonstrated similar cellular constituents in seventeen of nineteen patients examined. In these seventeen patients the infiltrate consisted of mature, peripheral T cells (Leu1/Lyt2^{+}, Leu5/Lyt3^{+}, Leu4^{+}, OKT6^{-}) admixed with Langerhans cells/indeterminate cells (HLA-DR^{+}, OKT6$^{+/-}$) (Figs 2 and 3). The latter cell types constituted a considerable proportion of the dermal infiltrate, and ranged from 20% to 50% of the dermal cells. Dendritic reticulum cells (R4/23^{+}, To5^{+}) were not identified in the biopsies from any of these seventeen patients. Occasional B cells (B1^{+}, To15^{+}) were present in between

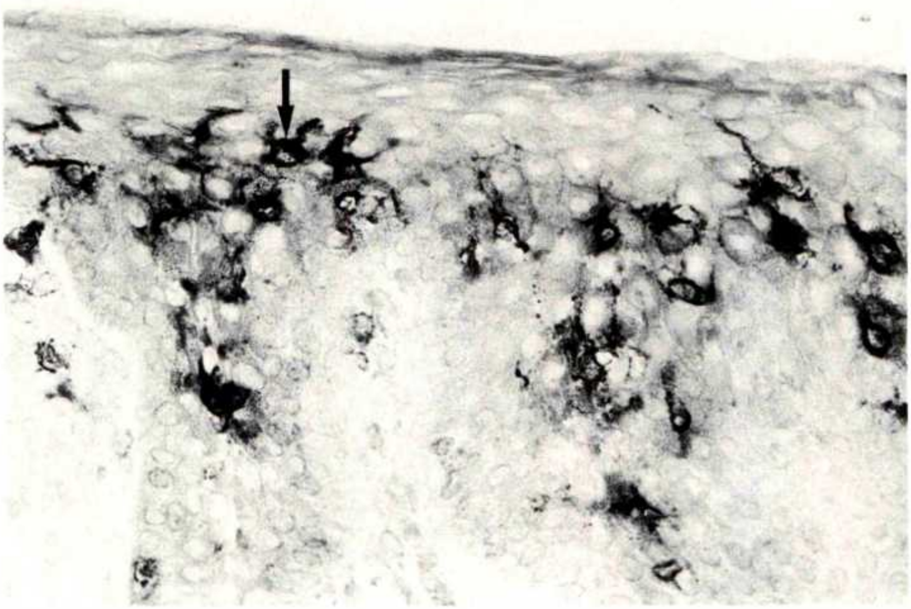

FIGURE 1. Immunoperoxidase labelling of skin biopsy with OKT6 (× 400) for the demonstration of Langerhans cells. A cell was scored as positive if the nucleus was clearly associated with dendrites (arrow). In this field a total of ten such cells are present.

the T cells in the dermal infiltrate in the biopsies from nine of the seventeen patients (Table 1: Nos 1, 2, 4, 5, 6, 9, 11, 12 and 15). However, the B cells constituted less than 1% of the dermal cells. B cells were not demonstrated in the epidermis, and were absent in the skin biopsies from the other eight patients (Table 1: Nos 3, 7, 8, 10, 13, 16, 17, and 19).

A different pattern was found in skin biopsies from the positive patch tests from the remaining two patients (Table 1: Nos 14 and 18). Thus in both of these patients aggregates of B1- and To15-positive B cells were present in the dermal infiltrate (Fig. 4a). The B cells were accompanied by R4/23 and To5-positive dendritic reticulum cells (Fig. 4b) forming B-cell follicles indistinguishable from those of peripheral lymphoid tissue. In both patients the B-cell follicles were surrounded by a mixed cellular infiltrate of mature peripheral T cells (Leu1/Lyt2$^+$, Leu5/Lyt3$^+$, Leu4$^+$, OKT6$^-$) (Fig. 4c), and Langerhans cells/indeterminate cells (HLA-DR$^+$, OKT6$^{+/-}$).

The two patients demonstrating B-cell follicles in their dermal infiltrate were sensitized to neomycin and PPD-mix respectively. In both patients biopsies were obtained relatively late, i.e. day 5 and 6 after epicutaneous exposure to the sensitizing reagent (Table 1). However, examination of five other biopsies from positive patch tests 5 days after exposure to the same (Table 1: Nos 13 and 17), or other sensitizing reagents (Table 1: Nos 12, 15 and 16) failed to reveal B-cell follicles in the dermal infiltrate. Thus no correlation was demonstrated between the presence of B-cell follicles, the sensitizing allergen, the extent of local reaction, or the time from application of allergen to examination.

The relative proportion of T-helper (Leu3$^+$) and T-suppressor (Leu2$^+$) cells in the biopsies from the nineteen patients is schematically outlined in Figure 5. The ratio between T-helper and T-suppressor cells varied considerably, and neither demonstrated correlation with any of

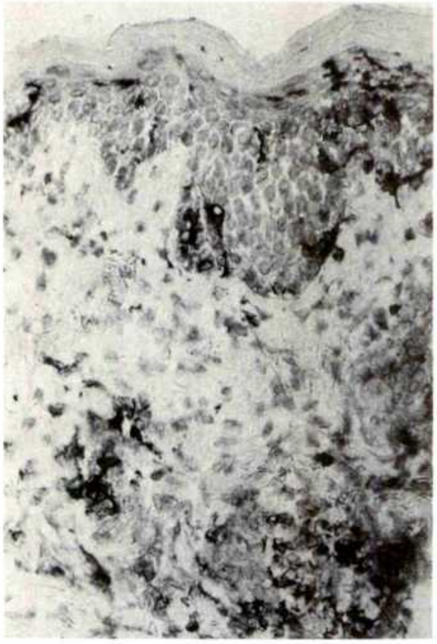

FIGURE 2. Immunoperoxidase labelling of biopsy from positive patch test with OKT6 (× 140) demonstrates Langerhans cells in the epidermis and dermis.

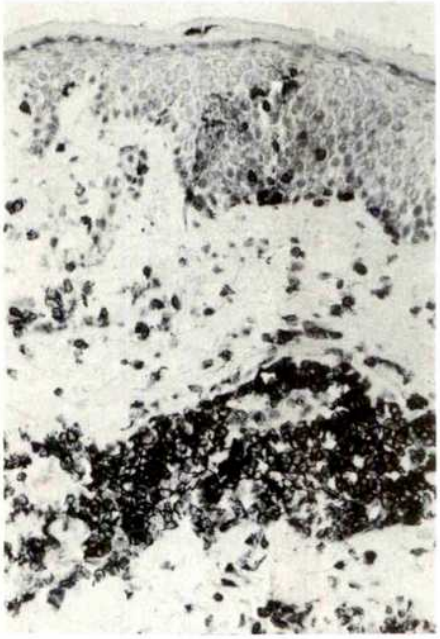

FIGURE 3. Immunoperoxidase labelling of the same case as in Fig. 2 with Leu4 (× 140) for the demonstration of T lymphocytes.

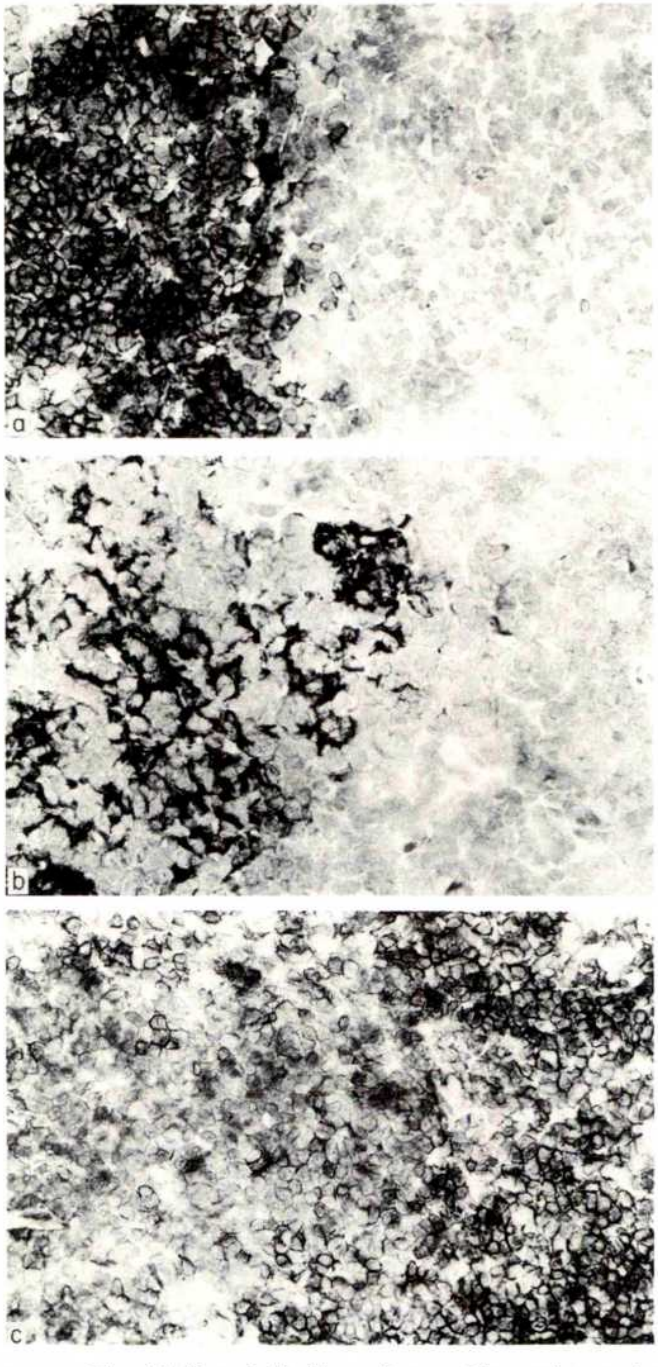

FIGURE 4. Immunoperoxidase labelling of skin biopsy from positive patch tests demonstrates (a) B lymphocytes (TO15, × 350) accompanied by (b) dendritic reticulum cells (R4/23, × 400) in the dermal infiltrate. These cells are surrounded by (c) T lymphocytes (Leu4, × 400), and a few T cells are also present in between in B cells. This organization of cells closely corresponds to that of peripheral lymphoid tissue.

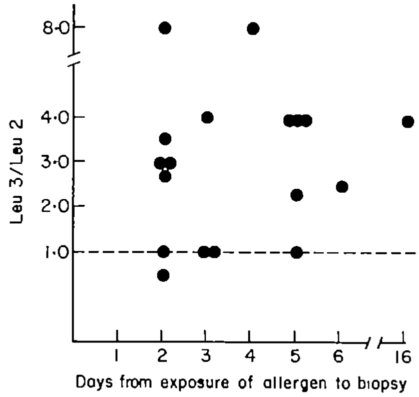

FIGURE 5. Schematic outline of the ratio of T helper (Leu3$^+$)/T suppressor (Leu2$^+$) lymphocytes in infiltrates from positive patch tests, compared with the time from application of allergen to examination (2–16 days).

the above mentioned variables. Furthermore the two subsets of T cells showed a similar distribution in all the biopsies examined. Both cell types infiltrated the epidermis. In addition both T-helper and T-suppressor cells were diffusely interspersed amongst the cells in the dermal infiltrate. The expression of 3A1 paralled the expression of Leu3 in all biopsies.

The T lymphocytes in the skin biopsies from all nineteen patients expressed HLA-DR antigen. Transferrin receptors (OKT9$^+$) were identified on subsets of HLA-DR antigen positive dendritic dermal cells (indeterminate cells). Furthermore transferrin receptors were present on subsets of the infiltrating T cells in biopsies from nine patients (Table 1: Nos 2, 3, 9, 10, 11, 12, 13, 18 and 19). T10 was not expressed by the infiltrating cells in either of the biopsies examined. Monocytes (MO2$^+$) and natural killer cells (Leu7$^+$) were not identified in the cellular infiltrate.

The number of OKT6-reactive epidermal Langerhans cells in positive patch tests and normal skin is detailed in Table 3. The number of Langerhans cells varied considerably from patient to patient. However, the mean number of Langerhans cells was slightly increased in positive patch

TABLE 3. Enumeration of OKT6-reactive epidermal Langerhans cells

Specimens	Age of lesion (d)	No. of samples	Number of Langerhans cells mean	range
Positive patch tests	2	7	52	29–79
	3	3	41	40–42
	4	1	42	—
	5	6	40	23–52
	6	1	46	—
	16	1	20	—
Normal skin	—	6	26	13–43

tests as compared with normal skin. The Langerhans cells also expressed HLA-DR. Furthermore, deposits of HLA-DR antigen were present between the keratinocytes in six patients (Table 1: Nos 1, 9, 10, 12, 16 and 19).

DISCUSSION

In this study frozen sections of biopsies from positive patch tests from nineteen patients have been analysed by immunoenzymatic labelling with a series of monoclonal antibodies. Two different patterns of reaction were identified. In seventeen patients the cells in skin infiltrates were T cells admixed with Langerhans cells/indeterminate cells. In two patients the dermal infiltrate also contained B-cell follicles indistinguishable from those in nodal lymphoid tissue.

The T cell/Langerhans cell pattern of infiltration corresponds to that recently described in smaller series of patients (Bruynzeel *et al.*, 1983; Haynes, Hensley & Jagosothy, 1982; MacKie & Turbitt, 1983). Experimental and *in vitro* functional studies have also firmly established the significance of Langerhans cells in the initiation and maintenance of contact allergic reactions (Toews *et al.*, 1980). It is generally agreed that this reflects antigen-presenting properties of Langerhans cells in the expansion of antigen-specific T lymphocytes (Stingl *et al.*, 1978). The participation of Langerhans cells in the pathogenesis of cutaneous contact allergy is thus further supported by this study.

B-cell follicles have not previously been described in positive patch tests. The specificity of anti-dendritic reticulum cell R4/23 is well documented (Naiem *et al.*, 1983). In particular R4/23 do not react with lymphocytes or Langerhans cells/indeterminate cells (Naiem *et al.*, 1983; Ralfkiær *et al.*, 1984). Furthermore the R4/23 positive cells in this study also reacted with anti-C3b-receptor To5. This is further evidence that the R4/23 positive cells in the biopsies from this study are dendritic reticulum cells (Gerdes & Stein, 1982b; Ralfkiær *et al.*, 1984).

Dendritic reticulum cells are important accessory cells in the generation of B-cell memory, and normally these cells localize in B-cell dependent follicular areas of peripheral lymphoid tissue (Gerdes & Stein, 1982b; Klaus *et al.*, 1980). Dendritic reticulum cells are not present in normal skin (Ralfkiær *et al.*, 1984). Furthermore, dendritic reticulum cells are very seldom present in benign and malignant lymphocytic infiltration of the skin, as shown by the absence of R4/23 positive cells in more than eighty skin biopsies from cutaneous T-cell lymphoma, psoriasis, lichen planus, and atopic dermatitis (Ralfkiær *et al.*, unpublished data). Recently, however, dendritic reticulum cells were identified in the skin infiltrates from some cases of B-cell lymphoma as determined by cytochemical criteria (Willemze *et al.*, 1983). Dendritic reticulum cells may thus under certain circumstances be attracted to infiltrate the skin, which suggests that dendritic reticulum cells are migratory cells. This concept is supported by the demonstration that antibody against dendritic reticulum cells also recognizes subsets of leukocytes in peripheral blood (Parwaresch *et al.*, 1983).

The demonstration of B cells accompanied by their relevant accessory cells in the skin infiltrates from positive patch tests suggests an involvement of B cells in the pathogenesis of cutaneous contact allergy. The presence of B-cell interacting dendritic reticulum cells in the skin further indicates that B-cell memory may, under certain circumstances, develop in extranodal lymphoid tissues.

In this study no correlation was demonstrated between the age of the positive patch test (2–16 days), the emergence of B-cell follicles or the ratio between T-helper and T-suppressor cells. This does not exclude the possibility that a sequence of recruitment of different subsets of lymphocytes and accessory cells may occur during the evolution and healing of positive patch tests in the individual patient (Bruynzeel *et al.*, 1983).

The identification of deposits of HLA-DR antigen between the keratinocytes from some of our patients is in accordance with results from other studies (MacKie & Turbitt, 1983). At present it is uncertain whether this reflects loss and subsequent diffusion of HLA-DR antigen from other cells to the keratinocytes, or induction of expression of HLA-DR on the keratinocytes (Tjernlund, 1980). The lack of uniformity in the presence of keratinocyte-associated HLA-DR antigen in this study may favour the first hypothesis. Both Langerhans cells/indeterminate cells and T lymphocytes may form sources of shedding of HLA-DR antigen. The T cells in the skin infiltrate from positive patch tests in this study thus all expressed HLA-DR antigen. This may reflect a state of cellular activation of the T lymphocytes (Reinherz *et al.*, 1979). This suggestion is supported by the identification of transferrin receptors on the T cells in the skin infiltrates from some of the patients in the present report (Terhorst *et al.*, 1981).

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Levels of arachidonic acid and its metabolites in the skin in human allergic and irritant contact dermatitis

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SUMMARY

Increased concentrations of arachidonic acid and prostaglandin E_2, but not 12-hydroxy-eicosatetraenoic acid, were found in the skin in human contact dermatitis due to nickel and chromate allergens. Significant levels of neutrophil chemokinetic activity, with similar properties to leukotriene B_4, were found in a high proportion of exudates from inflamed skin treated with allergen but not in exudates from untreated skin. Neither arachidonic acid nor its metabolites were increased in primary irritant dermatitis due to benzylalkonium chloride.

We previously reported that perfusates from the involved skin of patients with allergic contact dermatitis contained smooth muscle-contracting activity with the pharmacological and chromatographic properties of prostaglandins E and F (Greaves & Sondergaard, 1970; Greaves, Sondergaard & McDonald-Gibson, 1971). Subsequently, prostaglandin-like activity was detected in perfusates from inflamed skin due to experimental primary irritant dermatitis in seven of thirteen subjects (Sondergaard, Greaves & Jorgensen, 1974).

Besides metabolism to prostaglandins and thromboxanes by the cyclo-oxygenase pathways, arachidonic acid is oxygenated by several lipoxygenase enzymes to give a number of monohydroxy-eicosatetraenoic acids (HETEs), dihydroxy-eicosatetraenoic acids (diHETEs) and the leukotriene components of slow-reacting substance of anaphylaxis. Many lipoxygenase products have biological activity. The diHETE 5,12-dihydroxy-eicosatetraenoic acid (leukotriene B_4, LTB_4) has potent neutrophil chemokinetic properties (Ford-Hutchinson *et al.*, 1980; Palner *et al.*, 1980). The HETEs are weaker chemokinetic agents than LTB_4 (Goetzl *et al.*,

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1980) but 12-HETE has been found in high concentrations in diseased human skin (Hammarstrom *et al.*, 1975; Camp *et al.*, 1983b). Several leukotrienes are pro-inflammatory in human skin (Camp *et al.*, 1983a).

The role of lipoxygenase products and their interaction with prostaglandin metabolites in mediating skin inflammation is not clear. We have therefore measured levels of arachidonic acid, prostaglandin E_2 (PGE_2) and several lipoxygenase transformation products of arachidonic acid in involved skin due to allergic and primary irritant contact dermatitis.

METHODS

Subjects

Ten patients with patch test proven allergic contact dermatitis were studied. Nine were allergic to nickel and one to chromate. The mean age was 30 years (range 17 to 53 years) and there were nine females. Patients had little or no active dermatitis at the time of study. Primary irritant dermatitis was studied in five healthy volunteer male subjects. The mean age was 41 years (range 21 to 60 years).

Induction of contact dermatitis

Patients and volunteers gave informed consent, and the approval of the Hospital Ethical Committee was obtained for the experiments. Patients allergic to nickel or chromate were selected, according to the clinical evaluation of the degree of hypersensitivity, so as to obtain confluent erythema involving the whole site of application with some oedema and papules but no vesiculation or pustulation.

Nickel sulphate and potassium dichromate were formulated in yellow soft paraffin to concentrations of 1% and 0·25% respectively. The allergen (0·5 g) was applied to the surface of Lyofoam (Ultra Laboratories Ltd.) held in circular plastic wells, 35 mm diameter × 5 mm deep. The wells were then taped to clinically normal abdominal skin to give close 'skin-to-allergen' contact. A second well containing 0·5 g yellow soft paraffin and a third well with Lyofoam alone were applied as controls. The wells were removed at 24 h, the skin gently cleaned with soap and water and skin exudate obtained as described below.

Primary irritant dermatitis was evoked by an aqueous solution of benzalkonium chloride applied to the foam-filled plastic wells described above. Initially a dose inflammatory response curve was constructed with 0·5, 1·0, 2·5, 5·0 and 10·0% benzalkonium chloride. The concentration selected was that producing confluent erythema and oedema with papules but without vesiculation or pustulation. This proved to be either 2·5 or 5·0% in the five subjects studied. No volunteer showed a reaction to 0·5% benzalkonium chloride. Control cells contained either water or foam inserts alone. All wells were removed at 12 h and skin exudates obtained 4 h later when the reaction was most intense.

Inflammatory exudate

Suction bullae containing exudate were produced as previously described (Black *et al.*, 1977) on inflamed, vehicle control and untreated areas of abdominal skin. Each 0·1 ml sample was equilibrated with deuterated internal standards for mass spectrometry: 200 ng [5,6,8,9,11,12,14,15-^{2}H]arachidonic acid and 25 ng each of [3,3′,4,4′-^{2}H]PGE_2, [5,6,8,9,11,12,14,15-^{2}H]5-HETE and [5,6,8,9,11,12,14,15-^{2}H]12-HETE. Exudates were stored at −20°C until analysed.

The exudates were acidified to pH 3·5 with acetic acid and the arachidonic acid metabolites extracted into ethyl acetate. The solvent was removed under a stream of nitrogen and the lipid residue resuspended in 0·5 ml 0·1 M phosphate buffer, pH 8·4, and partitioned twice against 0·7 ml chlorobutane to extract arachidonic acid and the HETEs. The aqueous phase was acidified to pH 3·5 and extracted with ethyl acetate to recover LTB_4-like material and prostaglandins. The chlorobutane residues were treated with 0·05 ml ethereal diazomethane containing 10% methanol for 10 min and the resulting methyl esters chromatographed on a LiChrosorb-Si60 column × 4 mm, eluted with hexane:propan-2-ol:methanol 100:1:0·5 by volume at 1·5 ml/min. Fractions corresponding to the methyl esters of arachidonic acid, 12-HETE and 5-HETE were collected. Methyl arachidonate was further purified by reverse-phase HPLC (Hawk *et al.*, 1983). The ethyl acetate extracts, containing the more polar material, were chromatographed on a LiChrosorb-Si60 column, 250 × 4 mm, eluted with chlorobutane:acetonitrile:methanol:acetic acid 60:40:3:0·3 by volume and fractions corresponding to LTB_4 and PGE_2 collected.

Arachidonic acid, 12-HETE, 5-HETE and PGE_2 were assayed by gas chromatography–mass spectrometry (GC–MS). The conditions used for arachidonic acid and PGE_2 were as previously described (Hawk *et al.*, 1983). The 12-HETE and 5-HETE samples were dissolved in 25 μl N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and heated to 50°C for 30 min to give the respective trimethylsilyl ether–methyl ester derivatives. The 12-HETE samples were chromatographed on a 12·5 m × 0·3 mm OV-17 capillary column, 5-HETE derivatives on a 1 m × 2 mm 1% OV-101 column, both at 210°C with helium as the carrier gas. The mass spectrometer and operating conditions were as previously described (Hawk *et al.*,1983). The ions monitored for 12-HETE were m/z 295/301 and for 5-HETE m/z 406/414.

HPLC fractions corresponding to authentic LTB_4 were taken to dryness and redissolved in 0·3 ml Eagle's minimal essential medium buffered to pH 7·4 with 20 mM HEPES buffer. Each was analysed for LTB_4 equivalents by the agarose microdroplet chemokinesis assay (Smith & Walker, 1980) using human leukocytes (70–80% neutrophils) prepared as previously described (Camp *et al.*,1983b). The chemokinetic acitivity in test samples and HPLC solvent control

TABLE 1. Concentrations (ng/ml, mean ± s.e.m.) of arachidonic acid, PGE_2 and 12-HETE in control and inflamed skin 24 h after application of nickel or chromate allergen

	Control	Vehicle	Allergen
Arachidonic acid ($n = 10$)	2390 ± 210	2476 ± 209	4144 ± 410 *$P = 0·001$ †$P = 0·003$
PGE_2 ($n = 10$)	14·0 ± 2·3	15·8 ± 2·1	27·2 ± 2·1 *$P = 0·001$ †$P = 0·018$
12-HETE ($n = 10$)	17·2 ± 3·0	15·3 ± 1·3	24·5 ± 5·5

Control = empty well.
Vehicle = yellow soft paraffin.
* Paired *t*-test against control.
† Paired *t*-test against vehicle.

samples was assayed directly against standard LTB_4 dose-response curves. The results of the LTB_4 assay are not corrected for losses during extraction and purification. Recovery of LTB_4 was generally 30–50%.

RESULTS

Allergic contact dermatitis

The mean concentrations of archidonic acid, 12-HETE and PGE_2 in exudates from inflamed and control skin sites are shown in Table 1.

There was a significant rise in arachidonic acid and PGE_2 at 24 h after applying antigen. However, 12-HETE levels were not significantly raised. The yellow soft paraffin vehicle had no detectable effect. The concentration of 5-HETE in individual 0·1 ml exudate samples was below

TABLE 2. Levels of LTB_4 equivalents (pg/0·1 ml exudate) in individual exudates from control and inflamed skin 24 h after application of nickel or chromate allergen

Patient	Control	Vehicle	Allergen
1	*	*	*
2	*	*	*
3	10	*	18
4	*	*	*
5	*	*	10
6	*	*	12
7	*	*	17
8	12	9	14
9	*	*	19
10	*	*	*

* Not detectable. (detection limit = 9 pg/0·1 exudate).

TABLE 3. Concentrations (ng/ml, mean ± s.e.m.) of arachidonic acid, PGE_2 and 12-HETE in control and inflamed skin 16 h after application of benzalkonium chloride

	Control	Water	Benzalkonium chloride
Arachidonic acid	2777 ± 377	2117 ± 380	2590 ± 412
PGE_2	12·3 ± 2·3	14·1 ± 4·5	19·3 ± 3·7
12-HETE	11·9 ± 4·4	23·8 ± 8·3	24·1 ± 6·2

$n = 5$.
Control = empty well.

the limits of detection by GC–MS. Consequently fractions from HPLC containing 5-HETE from either inflamed, vehicle-treated or control sites of nine patients were pooled before analysis by GC–MS. The mean concentrations found were 6·8, 5·4 and 3·6 ng/ml for inflamed, vehicle and control sites respectively.

The levels of LTB_4 in individual exudates are shown in Table 2. Detectable amounts were found in only three of the twenty exudates from yellow soft paraffin vehicle and untreated control sites whereas six of ten exudates from allergen-treated skin contained LTB_4-like material.

Primary irritant dermatitis

There was no significant change (paired *t*-test) in the concentrations of arachidonic acid, 12-HETE and PGE_2 in the inflamed skin of the five subjects at 16 h after application of benzalkonium chloride compared with control skin (Table 3). LTB_4 was below the detection limit for all samples. 5-HETE was not assayed.

DISCUSSION

The present findings both confirm and extend previous reports (Greaves & Sondergaard, 1970; Greaves, Sondergaard & McDonald-Gibson, 1971) of elevated levels of arachidonic acid metabolites in allergic contact dermatitis. In the earlier studies biological activity co-chromatographing with PGE_1 was found in approximately five-fold greater amounts in perfusates from inflamed skin 48 h after challenge with allergen compared with control non-inflamed skin. In the present study using the suction bulla method, the increase in PGE_2 at 24 h was more modest but nevertheless significant. Arachidonic acid, the precursor of prostaglandins, was also found to be raised at 24 h. The finding of increased neutrophil chemotactic activity co-chromatographing with LTB_4 24 h after antigen challenge is of especial interest since LTB_4 is a highly potent neutrophil chemo-attractant (Ford-Hutchinson *et al.*, 1980) and the histological findings in acute allergic contact dermatitis include accumulation of small numbers of neutrophil granulocytes as well as lymphocytes (Pinkus, 1954; Pinkus & Mehregan, 1976). These results should prompt attempts to establish quantitative concordance between LTB_4 levels and the population density of neutrophils in inflamed skin. It is also possible that PGE_2 and LTB_4 behave synergistically in human skin (Camp *et al.*, 1983a). The two mono-HETE lipoxygenase products were not significantly raised in the inflamed skin.

We were unable to demonstrate statistically significant changes in the concentrations of arachidonic acid, or the cyclo-oxygenase and lipoxygenase metabolites, in primary irritant dermatitis although a trend towards increased levels of PGE_2 in inflamed skin was seen. This contrasts with the results of an earlier study (Sondergaard *et al.*, 1974) in which increased PGE-like activity was found in seven of thirteen perfusates from inflamed skin treated with benzalkonium chloride. Pharmacological heterogeneity amongst patients with primary irritant dermatitis is a possibility. A comparison of the clinical and pharmacological actions of non-steroidal anti-inflammatory drugs with selective inhibitory actions on either the cyclo-oxygenase or lipoxygenase pathways of arachidonic acid metabolism in allergic and primary irritant dermatitis would be of interest.

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Analytical, ultrastructural, autoradiographic and biochemical studies on [^{3}H]dicarboxylic acid added to cultures of melanoma cells

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SUMMARY

Lentigo maligna and malignant melanoma can be treated by dicarboxylic acids (C_9 and C_{12}), which are competitive inhibitors of tyrosinase. We therefore studied the intracellular location and possible sites of action of dodecanedioic acid (C_{12}) in murine melanoma cells, using EM autoradiography and biochemical analysis of lipid extracts by HPLC. Significant levels of radioactivity were found in the mitochondria and in the nuclei but not in association with membranes of rough endoplasmic reticulum, Golgi-associated endoplasmic reticulum, or Golgi apparatus, and not in coated vesicles or melanosomes. Biochemical analysis revealed that the diacid underwent β-oxidation, which occurs only in mitochondria. The results suggest that the toxicity of dicarboxylic acids in melanoma cells is not related to anti-tyrosinase activity but may be due to interference with oxidoreductase enzymes in the mitochondria and possibly to inhibition of DNA synthesis in the nucleus.

Dicarboxylic acids (from C_6 to C_{13}) are competitive inhibitors of tyrosinase *in vitro* (Nazzaro-Porro *et al.*, 1979a). When topically applied as a 15–20% cream to cases of lentigo maligna and primary cutaneous melanoma, some (C_9 and C_{12}) have been shown to have a cytotoxic effect on the human abnormally active and malignant melanocyte (Nazzaro-Porro *et al.*, 1979a,b, 1980). This cytotoxic effect might be related to anti-tyrosinase activity. We have previously investigated this possibility, by analytical ultrastructural autoradiography on the intracellular localization of added [^{3}H]dodecanedioic acid (C_{12}) within normal human melanocytes in tissue culture (Breathnach *et al.*, 1983). Significant levels of radioactivity were found in the mitochondria and the nuclei, but not related to any organelles associated with synthesis and successive location of tyrosinase, such as rough endoplasmic reticulum (RER),

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Golgi-associated endoplasmic reticulum (GERL), Golgi vesicles, coated vesicles, or melanosomes. This indicated that the cytotoxic effect of dicarboxylic acids may not be primarily related to anti-tyrosinase activity, but possibly to anti-mitochondrial activity, and to an effect on DNA synthesis in the nucleus.

The present investigation extends the ultrastructural analytical autoradiographic study to Harding-Passey and Cloudman mouse melanomata in cell cultures in order to determine whether localization of the radioactive diacid within malignant melanocytes is similar to that in normal cells. In parallel experiments, samples of Cloudman melanoma, and of a human melanoma cell line (B0008 supplied by Prof. Rona MacKie) were submitted to biochemical analysis to determine whether the diacid had undergone metabolism within the cells during the incubation period.

METHODS

Cultures

Confluent cultures of B16 Harding-Passey and Cloudman S91 murine melanomas and B0008 human melanoma were grown. The basic culture media differed as the three melanomas grow best in different media. The basic media were the following.

(a) For the Harding-Passey cells, Eagle's MEM medium with Hank's salts (Gibco) and sodium bicarbonate to a final pH of 7·6.

(b) For the Cloudman cells, Williams E medium (Flow Laboratories) with 2·2 g/l sodium bicarbonate to a final pH of 7·2.

(c) For the B0008 human cells, RPMI 1640 (Flow) with 20 mM Hepes and Na HCO_3 to a final pH of 7·3 to 7·4.

All media contained 10% fetal calf serum, 2 mM glutamine, 100 i.u./ml penicillin and 100 μg/ml streptomycin. The cells were grown in 5 ml plastic petri dishes on Thermanox plastic coverslips (Lux Scientific Co.). The cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2, 95% air. The cultures were fed at intervals of 3–4 days.

Autoradiography

When confluent, the Harding-Passey and Cloudman cells were incubated with 33·3 μCi/ml [10,11-^{3}H]dodecanedioic acid (Amersham International) of high radiochemical purity (more than 96%, specific activity 25 Ci per mmol). The concentration of dodecanedioic acid was $1{\cdot}33 \times 10^{-6}$ M. One dish, without the addition of isotope, was used as a non-radioactive control. Incubation with the isotope was for 2, 5 (Cloudman only), 20 or 60 min. At the end of the incubation time the culture medium was removed, and the cells were washed with PBS and fixed for 5 min at room temperature in 2·5% glutaraldehyde in 0·2 M cacodylate buffer. They were then washed with buffer, post-fixed in osmium tetroxide for 10 min, and washed in four changes of distilled water followed by 70% ethanol for 10 min. At this stage, the cells were gently scraped off the coverslips using a rubber spatula and centrifuged in ethanol at 25 *g* to form a pellet. After completing the dehydration in absolute ethanol, the cells were embedded in Araldite.

Ultrathin sections of pale gold interference colour were cut. The sections were transferred to formvar-coated nickel grids and emulsioned with Ilford L-4 nuclear emulsion using the loop technique and left to expose at 4°C. Controls for positive chemography and latent image fading were carried out at the same time. The length of exposure was monitored using 1 μm sections emulsioned with Ilford K-2 emulsion, as these are adequately exposed in one-tenth of the time needed for ultrathin sections emulsioned with L-4. A good grain density was seen after 10 days and the ultrathin sections were therefore exposed for 100 days.

At least 500 grains were photographed for each incubation time and the micrographs were analysed using a modified version (Ward, Gloster & Harris, 1979) of the computer-based hypothetical grain analysis (Blackett & Parry, 1973, 1977; Parry & Blackett, 1976). This method compares the observed distribution of silver grains, as seen on the micrographs, with a hypothetical distribution which would be obtained if the radioactivity were randomly distributed throughout the cell. The observed distribution of grains on the micrographs is compared with the hypothetical distribution by a computer program incorporating a minimizing subroutine which gives us an estimate of the relative intensity of sources or sources per unit area for each organelle. The structures chosen for the analysis were: nucleus, mitochondria, melanosomes, 'cytoplasm', i.e. areas of cytoplasm where no organelles were seen, and 'other', i.e. all other intracellular organelles and inclusions such as RER, GERL and Golgi membranes. Extracellular areas were also included in the count to check that background levels were acceptable.

Biochemical analysis

The shortest and longest incubation times used for the autoradiography were used for the biochemical study. The Cloudman and human B0008 cells were incubated as before with 33·3 μCi/ml of [^{3}H]dodecanedioic acid for 2 or 60 min. At the end of the incubation time, the medium was removed and replaced with phosphate buffer and the cells were then immediately scraped off the petri dishes, transferred to glass vials and extracted with chloroform–methanol 2 : 1 (v/v). The cells were left overnight at room temperature to extract the lipids present. The extract was then filtered and the solvent evaporated in a current of nitrogen. Aliquots of the cell lipid extract, and the residual matter containing proteins, carbohydrates, etc., were placed in counting vials in the presence of standard scintillation solution and counted by beta-ray spectrometry. The different fractions of lipid extract of cells (phospholipids, dicarboxylic acids, cholesterol, triglycerides, cholesterol esters) were obtained by thin-layer chromatography according to the method of Passi *et al.* (1981). Owing to the small amount of the starting material, only the dicarboxylic acid fraction was analysed by the HPLC–beta-ray spectrometry coupled system as follows.

Derivatization procedure. The dicarboxylic acid fraction was dissolved in 1 ml of CH_3–MeOH 4:1 (v/v). Then 0·2 mg of *p*-bromophenacylbromide dissolved in CH_3CN and 1 μl of the catalyst N,N-diisopropylethylamine were added. The mixture was heated to 50–60 °C for 10–15 min. Under these conditions, complete esterification of dicarboxylic acids to the corresponding, strongly chromophoric *p*-bromophenacyldiesters was achieved.

After evaporation of a part of the solvent, the esterified solutions were purified by TLC on standard thin-layer plates (Stratocrom SI-AP, Carlo Erba) coated with a 0·25 mm thick layer of silica gel and activated by heating at 120 °C for 20 min. The plates were developed by using benzene–hexane 3:1 v/v as the solvent system. The band corresponding to dicarboxylic *p*-bromophenacyldiesters (at the origin) was scraped off and extracted three times with 1 ml of CH_3CN. The solvent was evaporated down to 0·5 ml final volume.

The solutions (100 μl) were directly injected into a liquid chromatograph (1084 B, Hewlett-Packard) provided with a scanning spectrophotometer in the 190–540 nm wavelength range. The liquid chromatograph included an integrator giving peak and characteristic time for each peak in the chromatograms.

The separation of the dicarboxylic acids as *p*-bromophenacyldiesters was obtained on a reverse-phase column (25 cm × 4 mm i.d.) RP 18, 5 μm (Browlee Labs, St Clara) with the use of the UV absorbance detector operating at 255 nm. The chromatographic column was operated at 40°C. Mobile phase: an initial isocratic elution (60% CH_3CN in water adjusted to pH 3·10 with H_3PO_4) for 5 min followed by a gradient to 100% CH_3CN in 60 min. Flow rate: 1 ml/min; sensitivity to 256×10^{-4} absorbance units/cm (AU/cm); chart speed: 0·25 cm/min.

Quantification of radioactive dicarboxylic acids. Because of the small amount of radioactive dicarboxylic acids present in the samples, it was necessary to add standard non-labelled dicarboxylic acids as carrier. During the chromatographic run, both labelled and non-labelled dicarboxylic acids were individually collected, placed in counting vials in the presence of standard scintillation solution and counted by beta-ray spectrometry.

RESULTS

Autoradiography

The ultrastructural detail of the two cell types was well preserved (Figs 1–3) and there was no difference observed between the cells which had been incubated with the radioactive acid and non-treated control cells. Both cell types were poorly melanized.

Representative autoradiographs of both the Harding-Passey and Cloudman melanomas are shown in Figures 1 to 3. In both melanomas, grains can be seen concentrated over the

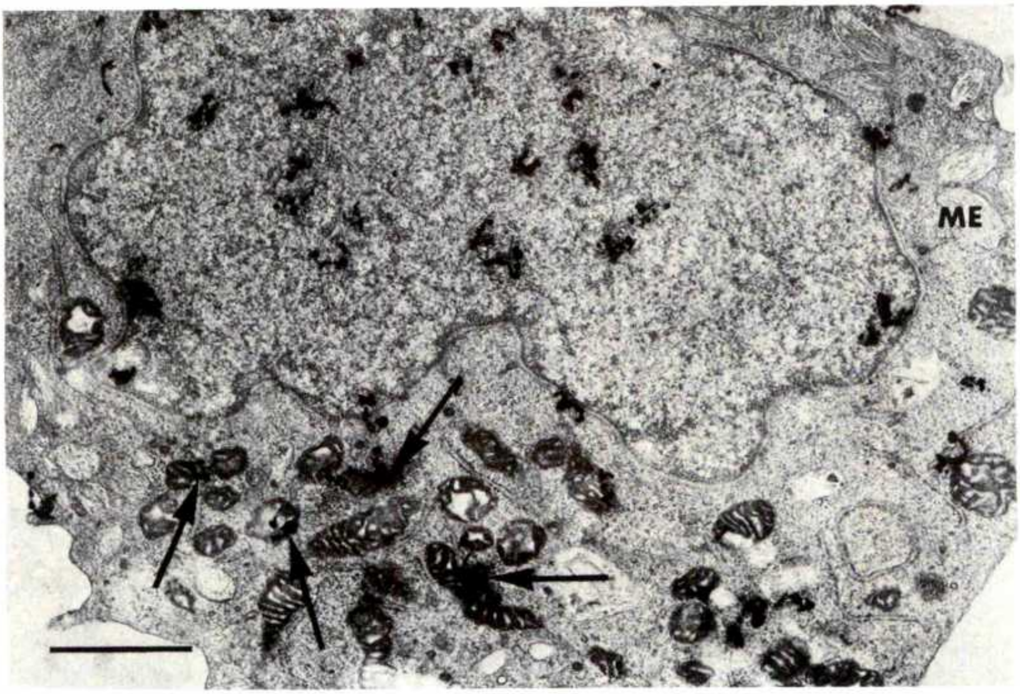

FIGURE 1. Autoradiograph of a cultured Cloudman cell fixed after 2-min incubation with [^{3}H]dodecanedioic acid showing silver grains over the nucleus and mitochondria (arrowed) but not over melanosomes (ME). Bar = 1 μm. (× 18,500.)

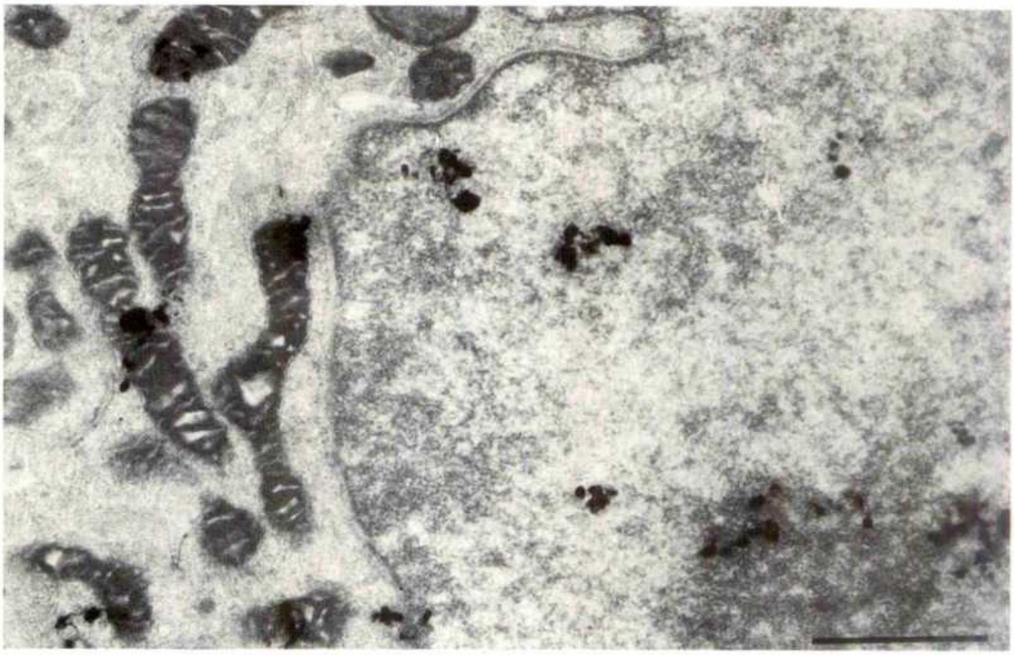

FIGURE 2. Autoradiograph of a cultured Harding-Passey cell fixed after 2-min incubation with [^{3}H]dodecanedioic acid showing grains over the nucleus and mitochondria. Bar = 1 μm. (× 23,000.)

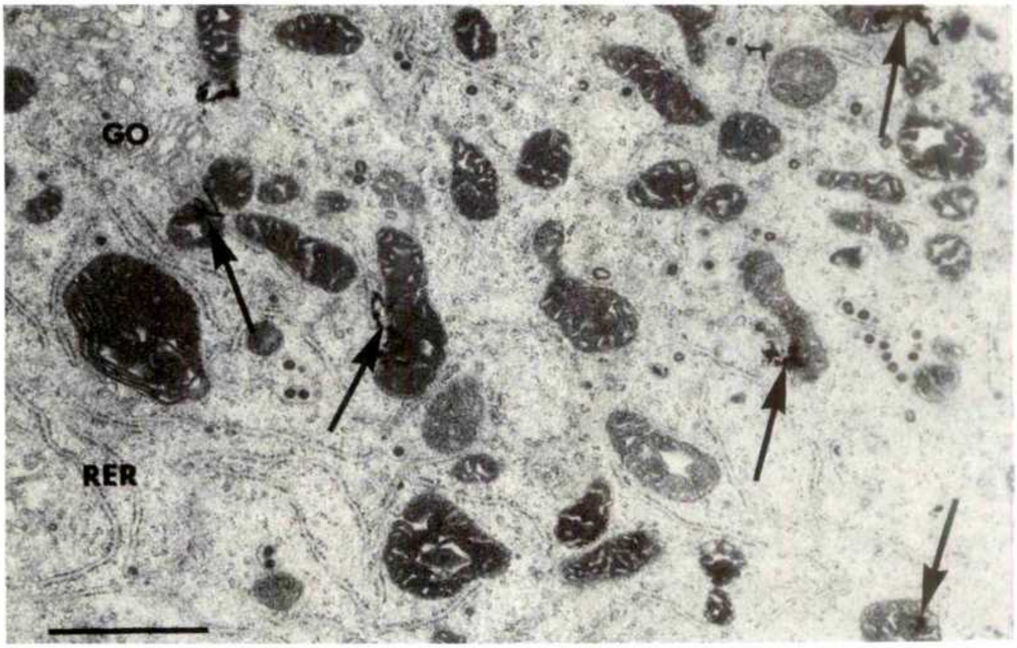

FIGURE 3. Autoradiograph of a cultured Harding-Passey cell fixed after 20-min incubation with [^{3}H]dodecanedioic acid showing grains over mitochondria (arrowed) but not over rough endoplasmic reticulum (RER) nor over Golgi membranes (GO). Bar = 1 μm. (× 21,000.)

TABLE 1. Relative intensity of sources in subcellular regions of Cloudman and Harding-Passey cells after different incubation times

	2 min	5 min	20 min	60 min
Cloudman				
Nucleus	0·22	0·43	0·45	1·10
'Cytoplasm'	0·07	0·19	0·20	0·37
Mitochondria	0·40	1·20	0·57	0·17
Melanosomes	0·05	−0·37	−0·26	0·31
Extracellular	0·11	0·08	0·17	0·29
Other	0·03	0·03	0·13	0·01
Harding-Passey				
Nucleus	1·41		1·05	0·23
'Cytoplasm'	0·31		0·29	0·07
Mitochondria	2·33		1·96	0·40
Melanosomes	−0·28		0·13	0·21
Extracellular	0·23		0·26	0·11
Other	0·17		−0·19	0·26

mitochondria and the nuclei. Several hundred similar autoradiographs were statistically analysed and the relative intensity of sources in the different regions of the cell is given in Table 1. Significant levels of radioactivity were found in the mitochondria and in the nuclei in both melanomas after only 2 min, and were significantly higher in both situations in the

TABLE 2. Percentage of radioactive dicarboxylic acids in lipid extracts of Cloudman and human (B0008) melanoma cells supplemented with [10, 11-^{3}H]dodecanedioic acid

Radioactive dicarboxylic acids	Radioactive C_{12} 1·33 × 10^{-6} M 2 min (%)	1 h (%)
Cloudman		
C_{12}	2·70	2·80
C_{10}	0·11	0·12
C_8	0·80	0·13
C_6	0·21	0·40
Human (B0008)		
C_{12}	2·50	2·60
C_{10}	0·15	0·20
C_8	0·17	0·20
C_6	0·20	0·38

Harding-Passey cells. In the Cloudman cells, the levels continued to rise in the nucleus while declining in the mitochondria during the 1-hour period studied. In the Harding-Passey cells, both mitochondrial and nuclear radioactivity declined from a high level after 2 min, with mitochondrial levels always higher than nuclear levels. Radioactivity in RER, Golgi apparatus, GERL, melanosomes and other organelles was not higher than background levels at any time in either melanoma.

Biochemical analysis

Only a small percentage (about 3%) of the total initial radioactivity was recovered in the dicarboxylic acid fraction, the remainder being present in other lipid fractions or non-lipid residual fractions which were not analysed in the present experiment. The results of the chromatographic analysis of the dicarboxylic acids in the lipid extracts themselves are given in Table 2. In the lipid extracts, 7–10% of the radioactivity was recovered in the dicarboxylic acid fraction and the remainder in the other lipid fractions represented by phospholipids, cholesterol and triglycerides. It is clear from all the samples of both tumour cell lines that not only was C_{12} diacid present, but also C_{10}, C_8, and C_6. The presence of the latter three establishes the fact that the C_{12} underwent β-oxidation within the melanoma cells.

DISCUSSION

This investigation shows that the basic pattern of incorporation of [^{3}H]dodecanedioic acid into Cloudman and Harding-Passey melanoma cells in culture is similar to that in normal human melanocytes (Breathnach *et al.*, 1983), the main targets again being the mitochondria and the nucleus, with little detectable incorporation of isotope into any intracellular organelles associated with tyrosinase location or melanogenesis. Analysis of the dicarboxylic acid fraction of the lipid extract of Cloudman and human B0008 melanoma cells showed the presence of labelled dicarboxylic acid catabolites with chain lengths 2, 4, or 6 carbon atoms shorter than 12, thus indicating that a β-oxidation with successive enzymatic removal of 2-carbon fragments from the molecule of the administered diacid was involved to some extent. This is further proof of the entry of dodecanedioic acid into mitochondria, since β-oxidation takes place exclusively within the mitochondrial matrix.

Recent experiments of ours (Passi *et al.*, 1983) showed that when orally administered to normal humans and rats, dicarboxylic acids are partly metabolized by β-oxidation. In other studies (Picardo *et al.*, 1983), we demonstrated that in isolated rat liver mitochondria, at higher concentrations (10^{-2} M) than that used in the present experiments ($1{\cdot}33 \times 10^{-6}$ M), dicarboxylic acids from C_8 to C_{13} interfere with the respiratory chain through competitive inhibition of mitochondrial oxidoreductases such as NADH dehydrogenase, succinic dehydrogenase, and reduced ubiquinone cytochrome C oxidoreductase. These enzymes are different from those such as acyl-CoA dehydrogenase, involved in β-oxidation. Stronger evidence is therefore accumulating to justify the suggestion that anti-mitochondrial activity may be a prime factor in the cytotoxic effect of dicarboxylic acids. This would fit in with Wilkie's (1978) proposition that some anti-cancer drugs may manifest their clinical usefulness primarily through anti-mitochondrial activity, and raises the possibility that dicarboxylic acids may have an effect on tumours other than melanoma. We are currently investigating this possibility.

The fact that radioactivity was also incorporated into the nucleus could indicate an effect of dicarboxylic acids on synthesis of nuclear DNA, although as previously pointed out (Breathnach *et al.*, 1983) it is possible that the nuclear radioactivity may be due to metabolites of the diacid which have passed from the mitochondria to the nucleus. The data in Table 1 do not allow us to say which is the more likely possibility. They do show, however, that in mitochondria of both Cloudman and Harding-Passey cells the radioactivity increased initially and decreased after longer incubation. After 1 hour's incubation, while ample isotope was still available in the medium, the level of radioactivity was not significantly higher than background level. Clearly, some change had occurred which blocked the uptake of the radioactive molecules into the mitochondria. At the same time, efflux of the isotope must have occurred or some radioactivity would still have been detectable. It is not easy to account for these changes in radioactivity within the mitochondria. They may concern progressive alterations in the mitochondrial membranes or interference with intermediates involved in transport of the diacid across the inner membrane.

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Effect of vitamin A and its derivatives on collagen production and chemotactic response of fibroblasts

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SUMMARY

Vitamin A and several other retinoids were added to fibroblast cultures in order to study possible alterations in biochemical properties and cellular responsiveness. The proliferation of cells was inhibited as the concentration of retinoids increased from 10^{-9} to 10^{-5} mol/l. Synthesis of non-collagenous proteins and production of both type I and type III collagen were decreased. The onset of type III collagen synthesis by tendon fibroblasts in culture was delayed. Furthermore, the chemotactic response of fibroblasts to fibroblast-conditioned medium was markedly reduced in the presence of retinoids (10^{-6} to 10^{-12} mol/l).

Vitamin A is known to influence the differentiation of ectodermal cells (Burgoon *et al.*, 1963). In the last few years, several derivatives of vitamin A have been developed which differ with respect to toxicity and pharmacological activity (Bollag, 1981). Some of these drugs have already been used for treatment of keratinizing disorders (Peck & Yoder, 1976), whereas others act mainly as anti-neoplastic agents (Bollag, 1979). Although a fair amount of information is available on the influence of vitamin A and its derivatives on the metabolism of ectodermal cells (Mellanby, 1947; Fell & Mellanby, 1952; Kochhar, 1973; Wilson & Reich, 1978; Shapiro & Mott, 1981), its effect on mesenchymal cells, e.g. fibroblasts, has not been investigated in great detail. However, the teratogenic activity of retinoids (Kochhar, 1973), the decrease in proliferation of fibroblasts (Lacroix, Anderson & Lippman, 1980) and the influence on mammalian collagenase (Brinckerhoff *et al.*, 1981) indicate an effect of vitamin A on these cells.

Fibroblasts synthesize collagen as the main structural glycoprotein of connective tissue (Layman, McGoodwin & Martin, 1971). It is known to be a polymorphous protein, which is present as several genetically distinct types (Bornstein & Sage, 1980). Skin fibroblasts produce

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simultaneously both type I and type III collagen in a constant ratio (Gay *et al.*, 1976), whereas freshly isolated tendon fibroblasts synthesize type I collagen exclusively (Uitto, Lichtenstein & Bauer, 1976).

Another characteristic feature of skin fibroblasts is their chemotactic response to preconditioned medium (Mensing *et al.*, 1983). Chemotaxis of fibroblasts plays an important role in situations as different as wound healing (Grinnell, Billingham & Burgess, 1981), fibrosis (Rennard *et al.*, 1981) and tumour invasion (Orr *et al.*, 1981). In the present study we were interested in determining whether the retinoids would affect these characteristic features of fibroblasts, namely synthesis of collagen and cellular responsiveness to environmental factors.

METHODS

Retinoids. All the retinoids [vitamin A, etretinate (Ro 10-9359), free acid of etretinate (Ro 10-1670) and isotretinoin (13-*cis*-retinoic acid)] were obtained as a gift from La Roche, Basel. Vitamin A and its derivatives were dissolved in 94% ethanol and then added to cell culture medium. All stock solutions were kept in the dark at $-70°C$.

Fibroblast cultures. Fetal human fibroblasts were obtained from fetal skin (28 weeks old) following a legal abortion. Adult human fibroblasts were grown from a biopsy of a healthy control (25 years old) as reported previously (Ihme *et al.*, 1983). Skin fibroblasts were seeded in low density (5×10^4 to 5×10^5 cells/ml) and retinoids were added to the medium. Chick tendon fibroblasts were freshly isolated using a routine procedure (Dehm & Prockop, 1971). All cells were kept as monolayer cultures and propagated as previously described (Krieg *et al.*, 1981).

Incubation and radioactive labelling of cells. In order to measure cellular proliferation, cells were harvested by trypsinization every second day and counted. Incubation with radioactive [^{3}H]proline was carried out in confluent monolayers in the presence of retinoids following a 3-day preincubation with retinoid-containing medium.

Tendon fibroblasts were freshly isolated from 16-day-old chick embryos and incubated after 24 hours. Another set of tendon cells was maintained in monolayers with and without retinoids and labelled after 5 days.

All incubations were carried out in Dulbecco's MEM medium containing 10% dialysed fetal calf serum, penicillin (400 U/ml), Na ascorbate (50 μg/ml) and [^{3}H]proline (10 μCi/ml).

Measurement of synthesis of collagen and non-collagenous proteins. The combined medium plus cell fragments was first dialysed against 1 mol/l $CaCl_2$, 0·05 mol/l Tris, pH 7·4, for 12 hours and then extensively dialysed against 0·5% acetic acid and subsequently lyophilized, hydrolysed, and analysed as previously described (Wiestner *et al.*, 1979).

Relative percentage of collagen synthesis was calculated according to the following formula:

$$\%\ \text{collagen} = \frac{2(\text{c.p.m. Hyp})}{5(\text{c.p.m. Pro} - \text{c.p.m. Hyp}) + 2(\text{c.p.m. Hyp})} \times 100$$

Characterization of collagen types. Newly synthesized collagens were treated with pepsin (Layman *et al.*, 1971) and collagen type I and type III were precipitated by 2·7 M NaCl. Subsequent chromatography on Agarose A 5m under denaturated conditions separated type III (gamma-components) from type I collagen (alpha-components). Following reduction with mercaptoethanol, type III collagen migrates as alpha-components (Krieg *et al.*, 1981). The relative amount of type III collagen to type I collagen was calculated from Agarose chromatograms.

Chemotactic activity. Embryonic human skin fibroblasts were incubated for 3 days in medium containing increasing concentrations of various retinoids. Their chemotactic response was then studied in a Blind well chamber (Fa. Biorad, Munich, F.R.G.). Polycarbonate filters (diameter 13 mm, pore size 8 μm; Nucleopore, Pleasonton, California, U.S.A.) were coated with gelatin (5 mg/l, Sigma) as described by Postlethwaite (1978). The lower compartment of the chamber was filled with the chemo-attractant (0.2 ml) and the gelatin-coated polycarbonate filter placed above.

Fibroblasts were harvested by short trypsinization (0·25% trypsin, 0·1% EDTA), suspended in DMEM containing 10% FCS for 5 minutes to inactivate the enzyme, centrifuged and resuspended in DMEM without FCS at a cell density of 4×10^5/ml. Aliquots of these cell suspensions (0·8 ml) were added to the upper compartment of the chamber. Conditioned DMEM medium from embryonic human fibroblasts cultures was used as chemo-attractant. Control cells and retinoid-treated cells were incubated for 4 hours at 37°C, 95% air/5% CO_2. The filters were then removed, fixed in ethanol and stained with toluidine blue. Cells which had migrated to the lower side of the filters were counted in a 400-fold magnification field under a Leitz microscope. Each sample was assayed in triplicate and cells were counted in five unit fields.

RESULTS

Cell growth and morphology

Retinoids were added in ethanol solutions to the culture medium. Equivalent ethanol concentrations were investigated and could be shown to be without any effect on cell growth and protein synthesis (not shown).

Addition of retinoids to fibroblast cultures during the exponential growth phase inhibited the growth rate of normal human skin fibroblasts, embryonic skin fibroblasts and freshly isolated chicken tendon fibroblasts (Fig. 1). This inhibition was concentration dependent and the slope

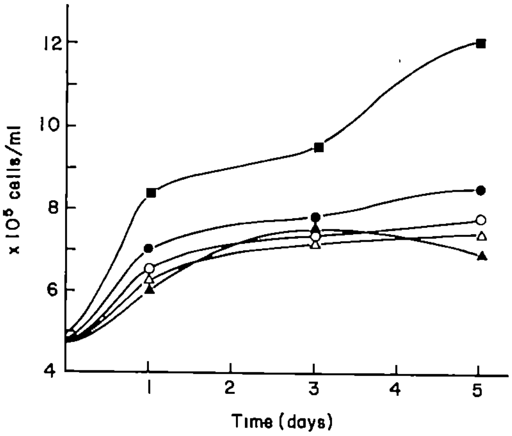

FIGURE 1. Growth rate of adult human skin fibroblasts in the presence of various retinoids (10^{-5} M). The cells were seeded at low density, and triplicate cultures were counted daily. (■) controls; (○) vitamin A; (△) 13-*cis*-retinoic acid; (▲) etretinate; (●) free acid of etretinate.

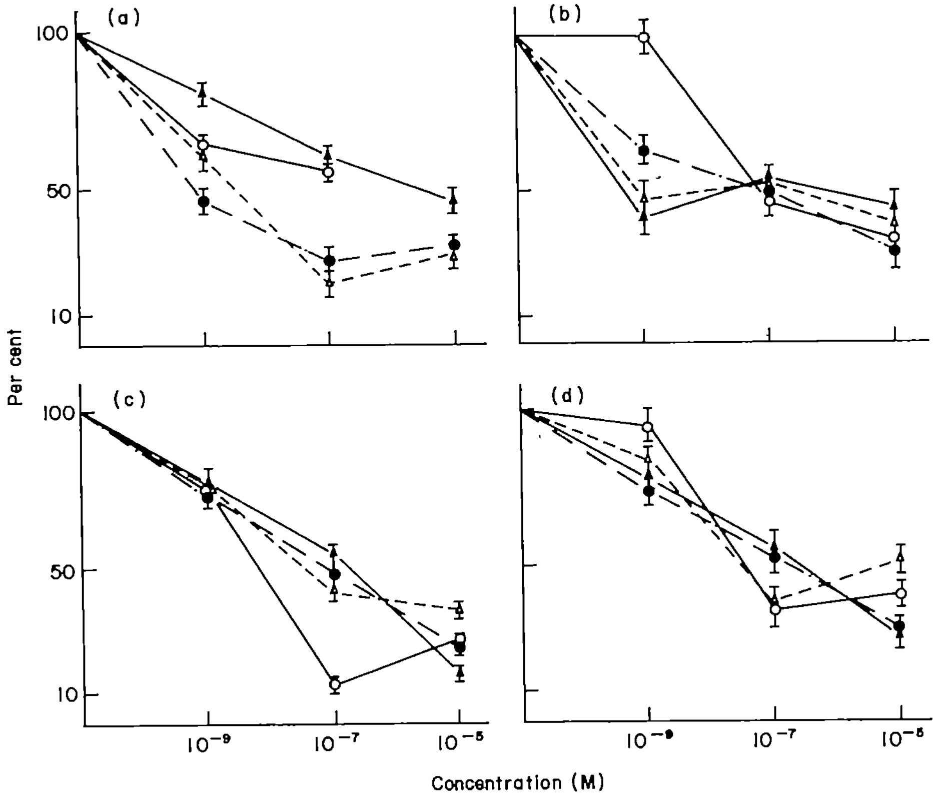

FIGURE 2. Synthesis of collagen (a–c) and non-collagenous proteins (d) in the presence of retinoids. The values are expressed as a percentage of the control values. (a) Adult human skin fibroblasts. (b) Freshly isolated chick tendon fibroblasts. (c) Embryonic human skin fibroblasts. (d) Non-collagenous protein synthesis in human embryonic skin fibroblasts. Synthesis of collagen and non-collagenous proteins was measured by labelled protein bound hydroxyproline and proline as described above in Methods. (○) vitamin A; (△) 13-*cis*-retinoic acid; (▲) etretinate; (●) free acid of etretinate.

was consistent. No alteration in cell morphology (checked by phase-contrast microscopy) or viability (Trypan blue) was noted using concentrations of retinoids varying from 10^{-9}–10^{-5} mol/l. The differences between the various retinoids were minor.

Synthesis of collagen and non-collagenous proteins

Production of collagen decreased with increasing concentrations of retinoids to about 25% of the controls (10^{-5} mol/l) in adult human skin fibroblasts (Fig. 2a) in embryonic human skin fibroblasts (Fig. 2c) and in freshly isolated chick tendon fibroblasts (Fig. 2b). The degree of inhibition of collagen production was similar for all retinoids studied and was dose dependent in all cases.

At low concentrations of retinoids (10^{-9} mol/l) synthesis of both collagen and non-collagenous protein were equally inhibited (Fig. 2d). As concentrations increased (10^{-5} mol/l), collagen production was slightly more affected than non-collagenous protein synthesis, resulting in a decrease of the relative amount of collagen from the total newly synthesized

TABLE 1. Relative proportion of collagen synthesis vs. non-collagenous proteins for (a) adult human skin fibroblasts; (b) embryonic human skin fibroblasts, and (c) freshly isolated chick tendon fibroblasts. The cells were kept for 3 days in the presence of various retinoids

Retinoids	Concentrations (M)	% Collagen from total protein (a)	(b)	(c)
Vitamin A	10^{-5}	3·8 ± 0·2	3·8 ± 0·3	5·3 ± 0·3
Isotretinoin (13-*cis*-retinoic acid)	10^{-5}	3·9 ± 0·2	4·0 ± 0·4	4·5 ± 0·3
Free acid of etretinate (Ro 10-1670)	10^{-5}	5·0 ± 0·4	1·1 ± 0·1	4·0 ± 0·2
Etretinate (Ro 10-9359)	10^{-5}	3·2 ± 0·3	4·7 ± 0·4	3·5 ± 0·3
Controls ($n = 9$)	0	5·2 ± 0·5	5·6 ± 0·4	6·4 ± 0·4

protein (Table 1). Even at 10^{-5} mol/l free acid of etretinate had no specific effect on collagen synthesis.

Synthesis of collagen types I and III

Pepsin-treated collagens from confluent cultures were salt precipitated and type I and type II collagens were separated by Agarose chromatography under denaturing conditions. Under

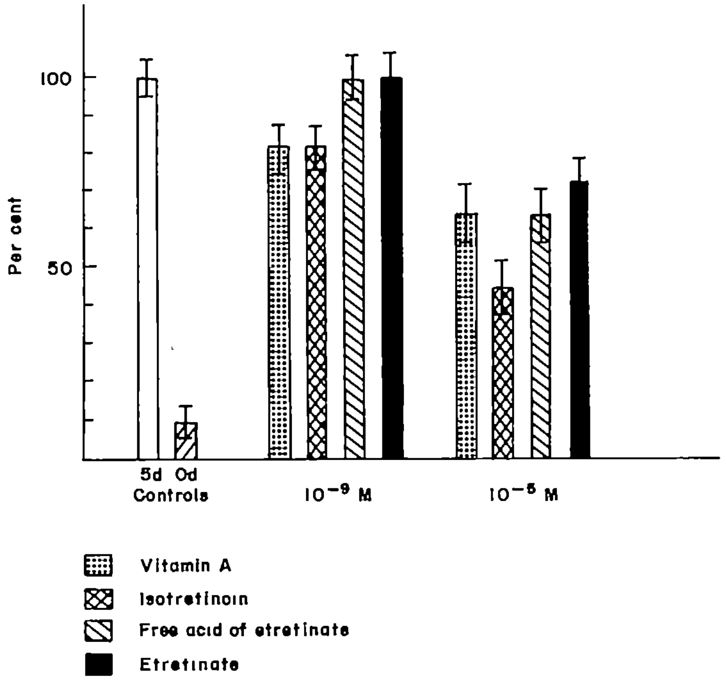

FIGURE 3. Influence of retinoids on type III collagen synthesis in freshly isolated chick tendon fibroblasts. Tendon fibroblasts were freshly isolated and seeded as monolayer culture in a high cell density. Retinoids were added daily to the culture medium (5 days).

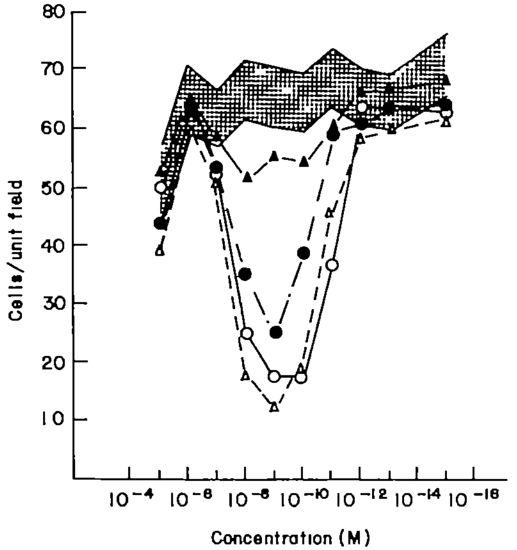

FIGURE 4. Influence of retinoids on chemotactic response of human skin fibroblasts. Cells were exposed to retinoids (10^{-12} M to 10^{-6} M) for 3 days prior to the experiment. (▨) controls; (○) vitamin A; (△) 13-*cis*-retinoic acid; (▲) etretinate; (●) free acid of etretinate.

reducing conditions type I collagen was found as alpha-component whereas type III collagen was identified in the region of gamma-components. In adult and embryonic human fibroblasts the ratio of type I to type III collagen was the same in both controls and cultures treated with retinoids (not shown).

Freshly isolated chick tendon fibroblasts were investigated directly after isolation and also after 5 days in monolayer culture. No type III collagen was synthesized by the freshly isolated cells. However, after several days in culture these cells produce considerable amounts of type III collagen. When various retinoids were added to freshly isolated cells, the production of type III collagen was suppressed to about 30% of the controls (10^{-5} mol/l) (Fig. 3).

Chemotactic activity

No chemotactic activity was found when vitamin A and its derivatives were used as chemo-attractants (not shown). When medium conditioned by embryonic fibroblasts was used as chemo-attractant, some 80–100 cells/unit field showed a chemotactic response. Another set of embryonic human fibroblasts were then preincubated with various retinoids for 3 days and subsequently investigated in a Blind well chamber containing conditioned medium as chemo-attractant. Pretreated cells (10^{-8}–10^{-10} mol/l) showed a marked decrease of chemotactic response (Fig. 4).

Surprisingly, no influence of retinoids was noted when lower or higher concentrations were used. With the exception of etretinate (Ro 10-9359), all retinoids studied had a similar effect on chemotaxis. Etretinate was less potent.

DISCUSSION

Logarithmic growth of fibroblasts was significantly inhibited only when high concentrations (10^{-5} mol/l) of retinoids were added to the cultures, as has been reported previously (Lacroix *et* *al.*, 1980). No obvious difference was noted when different retinoic acid derivatives were used, although toxicity of these components has been reported to differ in other systems (Bollag, 1981). Viability of the treated cultures was also corroborated by the normal morphology and the Trypan blue exclusion test.

Two different fibroblast activities, collagen synthesis and the chemotactic response, were then studied using vitamin A and various analogues (isotretinoin; free acid of etretinate; etretinate). Collagen production and the synthesis of non-collagenous proteins dropped with increasing concentrations in adult and embryonic human skin fibroblasts. From equivalent experiments using freshly isolated chick tendon fibroblasts it can be concluded that retinoids affect both chick and mammalian systems in a similar manner. In addition, fibroblasts grown from different organs showed a similar response to retinoids. There is circumstantial evidence that the effect of most retinoids studied is somewhat stronger on collagen than on general protein synthesis, when higher concentrations were used (10^{-5} mol/l). Several types of collagen are synthesized by fibroblasts, which are present in a certain ratio (Bornstein & Sage, 1980).

In a second set of experiments we therefore determined whether the decrease in collagen production could be due to the selective inhibition of one collagen type. However, the unaltered ratio of type I to type III collagen after retinoid treatment suggests that production of both type I and type III collagen is similarly decreased. This suggests that the same regulatory mechanism may control the synthesis of both collagen types.

Freshly isolated tendon fibroblasts are known to synthesize exclusively type I collagen and switch to the production of type I and type III collagen after a few days in monolayer culture (Herrmann *et al.*, 1980) (Fig. 3). Previous experiments indicated that this observation is not due to the selection of a particular population of fibroblasts but represents a modulation of the phenotype of the original cell population. Addition of retinoids maintains the original differentiated stage of freshly isolated tendon fibroblasts as indicated by the suppressed synthesis of type III collagen, even after several days of culture.

Chemotactic response of fibroblasts was chosen as a second system to investigate the influence of vitamin A and its derivatives on human skin fibroblasts. Retinoids themselves were shown to have no chemotactic activity; therefore chemotactic response was measured in cells under the influence of vitamin A and its derivatives during 3 days prior to the experiment. The decrease in chemotactic activity observed in the majority of cell types studied was clearly limited to a particular concentration, which was within therapeutic limits ($1{\cdot}7 \times 10^{-6}$ mol/l). The observation that high concentrations of retinoids do not affect chemotactic behaviour of the cells indicated that the alteration is not due to a general inhibition of cellular migration caused by a toxic effect of these compounds. This was furthermore corroborated by control levels of random migration with all concentrations of retinoids used.

Alteration of the several aspects of cellular metabolism which we have studied, namely decrease in proliferation and production of collagen and non-collagenous proteins and a reduced response to chemo-attractants, clearly show that vitamin A and its derivatives can influence mesenchymal cells. In most instances the differences between the various derivatives were minute. These data suggest that under certain conditions, such as wound healing, which involve fibroblast proliferation, migration and the deposition of connective tissue constituents, treatment with retinoids may affect these cellular functions.

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Basement membrane components outline the tumour islands in cylindroma

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SUMMARY

The main histological feature of cylindroma is the deposition of sheaths of a 'hyalinized' material contiguous to the tumour cell clusters. Although ultrastructural studies of this material have revealed a basement membrane-like structure, its exact nature has remained unclear. Using immuno-staining with affinity-purified antibodies directed against distinct basement membrane components, we have shown that type IV collagen and laminin are major constituents of this zone. In addition, cell culture studies indicated that both proteins are synthesized by the tumour cells. The immunohistological data make it clear that the tumour matrix between the tumour cell islands is composed not only of basement membrane components, but also is composed of other connective tissue constituents, i.e. type I and III collagen and fibronectin.

Cylindroma or Spiegler's tumour is an adnexoid tumour of indeterminate origin, which rarely undergoes malignant transformation. Histologically this tumour is characterized by epithelial cell clusters, which are encircled by a rim of 'homogeneous' material. Histochemical data (Braun-Falco, 1955) and electronmicrographic data (Gebhart, Kokoschka & Wick, 1975) have suggested a basement membrane-like structure of this zone. The definitive nature of its components is still unclear.

In recent years considerable progress has been made in the identification and characterization of basement membrane proteins. Specific antibodies against a variety of these components have been developed (Timpl & Martin, 1982). It was therefore the aim of our study to further characterize the 'homogeneous' material in cylindroma by using specific reagents to well-defined basement membrane components. For this purpose purified antibodies against type IV collagen, laminin, interstitial collagens type I and III, and against fibronectin were used in

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immunohistological studies. In addition, tumour cells were propagated in monolayer cell cultures for the characterization of their biosynthetic capacity.

METHODS

Specimens from three cylindromas obtained by surgical excision were examined. Routine histological sections stained with haematoxylin–eosin, Van Gieson, Masson's trichrome and Periodic acid-Schiff were prepared from each case. Cryostat sections were used for immunohistological examination. The previously described affinity-purified antibodies specific for types I and III collagen, fibronectin, type IV collagen and laminin were used (Timpl *et al.*, 1977, 1978, 1979).

Immunofluorescence procedures

Frozen, unfixed 4 μm thick sections were incubated with the purified antibodies diluted in phosphate-buffered saline (PBS), pH 7·2 (20–50 μg/ml). Subsequently the specimens were treated with goat anti-rabbit immunoglobin G conjugated with fluorescein isothiocyanate (FITC) (Behringwerke AG, Marburg, F.R.G.). After each incubation step the tissue slices were thoroughly washed with PBS. Normal rabbit IgG or PBS served as controls.

Microscopical examination was carried out using a Zeiss Standard 18 microscope equipped for incident light immunofluorescence (FITC filter combination BP 450–490, FT 510, LP 520–560). Photomicrographs were taken on Kodak Tri-X pan films.

Cell culture dishes containing attached epithelial cells were washed with PBS and fixed with 80% ethanol for 5 min to allow antibody penetration to extra- and intracellular antigens. Cell cultures were treated with hyaluronidase, 2 mg/ml PBS, pH 5 (Serva, Heidelberg, F.R.G.) to unmask antigenic determinants prior to staining with the specific antibodies.

Cell culture

Small pieces of the tumour were incubated with Hams F 10-Medium (Labor Service, Munich, F.R.G.) containing 0·2% collagenase (Worthington Biochemicals, Inc. Freehold, N.J.), 0·25% trypsin and 0·1% hyaluronidase at 37°C for 30 min to 2 h, in order to dissociate the cells from the surrounding matrix. After filtration through a nylon filter (20 μm pore size) and centrifugation (500 ***g*** for 5 min) the isolated cells were washed three times in PBS, pH 7·2, and seeded on to 3 cm plastic dishes (Becton and Dickinson, Heidelberg, F.R.G.) in Hams F 10-Medium supplemented with 10% fetal calf serum, sodium ascorbate (50 μg/ml), L-glutamine (300 μg/ml), penicillin (400 μg/ml) and streptomycin (50 μg/ml). Cells were kept in a moist atmosphere at 37°C in a CO_2 incubator (5% CO_2/95% air). The medium was changed on alternate days. Cell morphology and growth characteristics were checked by phase-contrast microscopy and documented on Tri-X pan films.

RESULTS

In each tissue specimen the clinical diagnosis was confirmed by conventional histological examination. PAS-staining revealed the typical 'hyalinized' sheaths surrounding the tumour islands (Fig. 1).

Incubation with anti-type I and III collagen antibodies revealed strong reactions to the collagenous part of the tumour stroma contiguous to the PAS-positive 'hyaline' sheaths (Fig. 2). Fibronectin could be seen uniformly distributed over the tumour stroma and at the periphery of

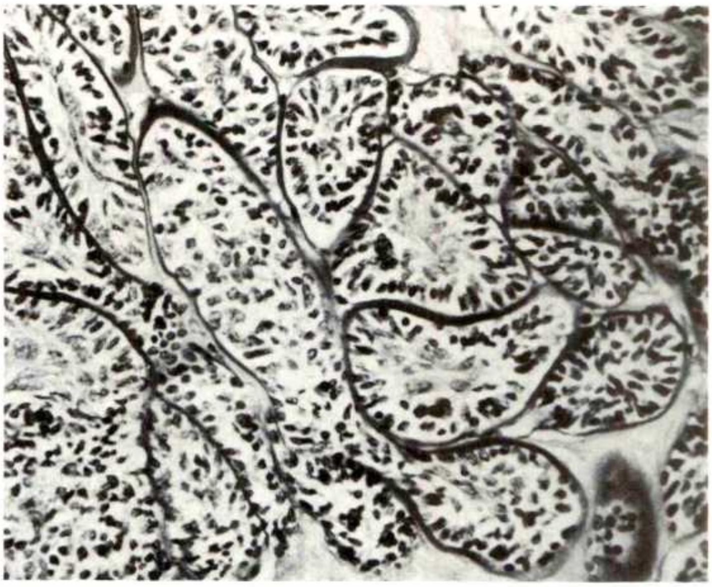

FIGURE 1. Cylindroma. Conventional histological section. PAS-staining shows the 'hyalinized' sheaths bordering the tumour cell clusters (× 230).

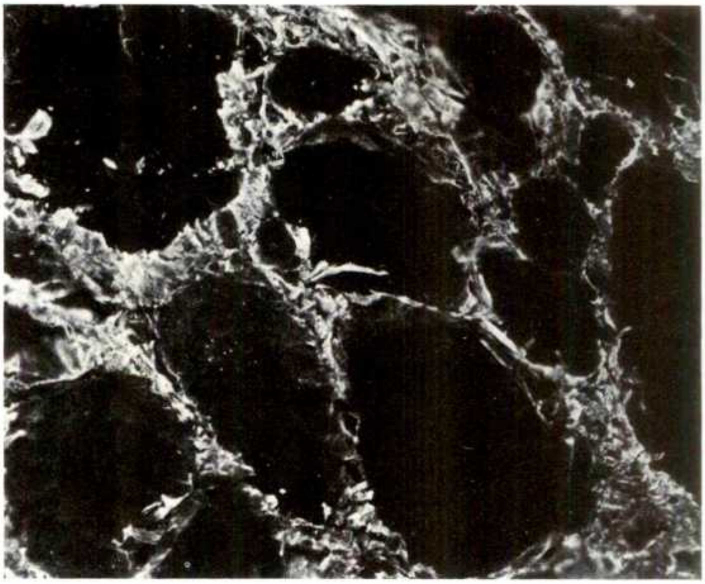

FIGURE 2. Cylindroma. Indirect immunofluorescence test with purified antibodies against type III collagen shows intense staining of the tumour stroma between the tumour cell islands (× 300).

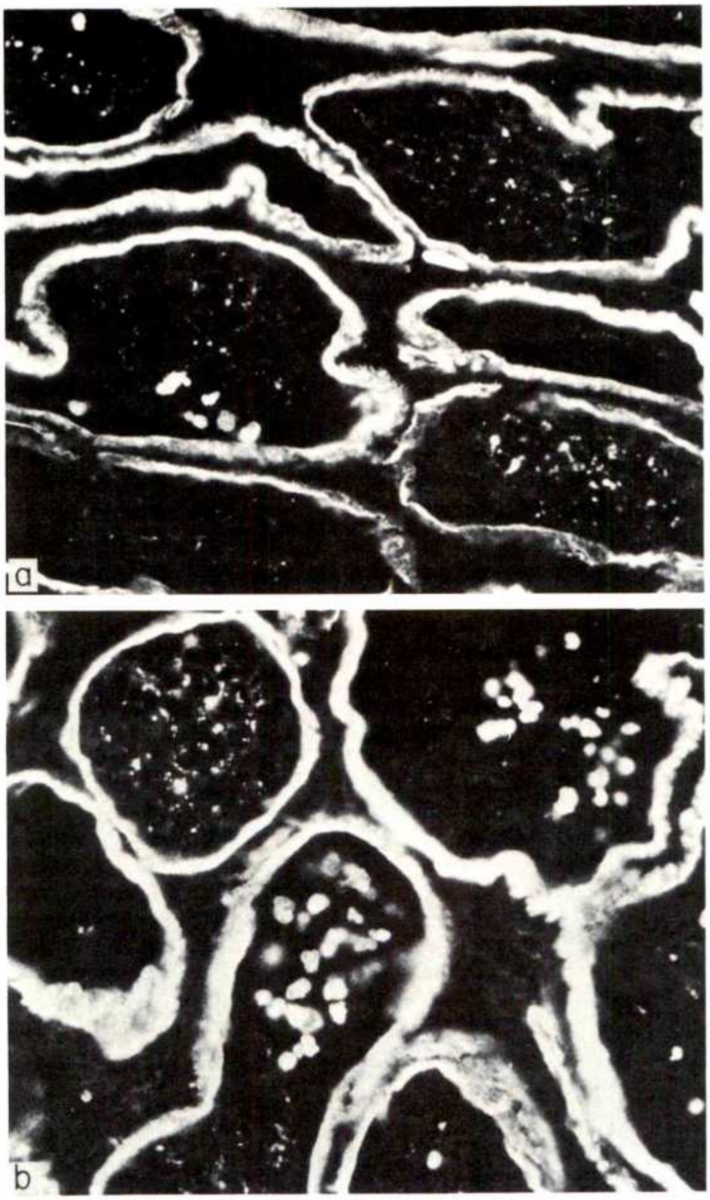

FIGURE 3. Cylindroma. Indirect immunofluorescence test with purified antibodies against type IV collagen (a) and laminin (b). Both proteins outline the tumour cell islands and are found as drop-like deposits within the tumour cell masses (× 300).

the cell islands (not shown). After staining with antibodies against type IV collagen and laminin there was a bright fluorescence. However, this did not cover the total area between the epithelial cell clusters, but was confined to a definitive zone encircling the tumour nests (Fig. 3a, b). Focal homogeneous areas of fluorescence corresponding to the PAS-positive 'globular' structures of cylindroma were also visible within the tumour cell masses (Fig. 3). Examination with the light microscope showed both proteins to be closely associated.

Cell cultures were established from two cylindroma samples after collagenase disaggregation of the tumour matrix. Most of the epithelial tumour cells displayed a honeycomb appearance in monolayer cell cultures, as visualized by phase-contrast microscopy (Fig. 4a). The synthetic activity of these tumour cells was examined by the indirect immunofluorescence technique. The cells showed negative staining for type I and III collagen, and little staining for fibronectin. In most of the tumour cells clearly positive reactions after staining with anti-type IV collagen and anti-laminin antibodies were seen. Fluorescent staining was predominantly confined to the intercellular space of the cell layer (Fig. 4b).

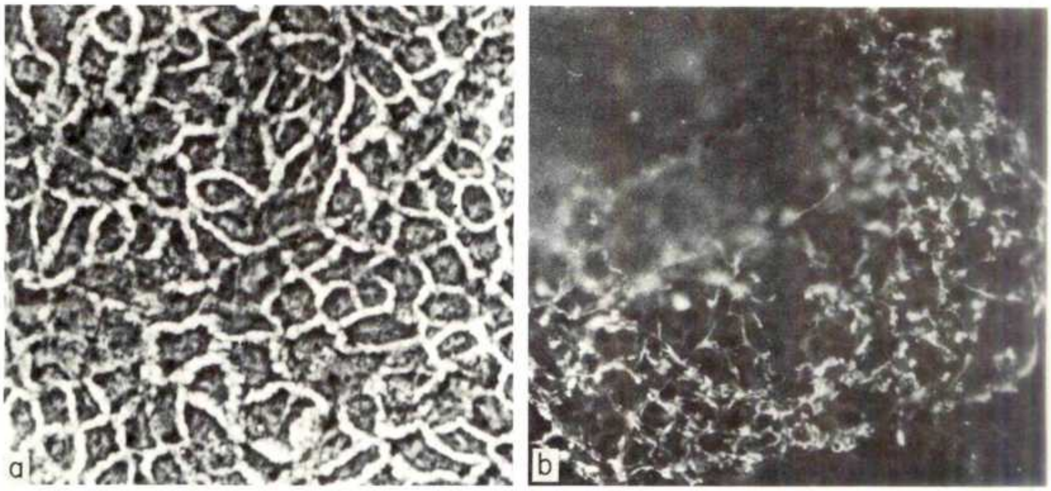

FIGURE 4. Monolayer cell culture of cylindroma tumour cells. (a) Phase-contrast microscopy demonstrates the honeycomb appearance of the cell layer (× 190). (b) Indirect immunofluorescence test with purified antibodies against laminin reveals intense staining of the intercellular space (× 120). The pattern of fluorescence is analogous to staining with antibodies against type IV collagen.

DISCUSSION

The main histological features of cylindroma are sharply demarcated epithelial cell clusters, which are encircled by a distinctive zone of material homogeneous in appearance. This PAS-positive substance has been characterized as glycoprotein by histochemical and biochemical studies (Braun-Falco, 1955; Siebert, Braun-Falco & Weber, 1955; Munger, Graham & Helwig, 1962). Electronmicrographic investigation has revealed extensive protrusions of the lamina densa as well as numerous anchoring filaments, indicating a basement membrane-like structure of the peritumoral rim and the intratumoral masses (Hashimoto & Lever, 1969; Gebhart *et al.*, 1975).

The staining with antibodies directed against laminin and type IV collagen is in agreement with this interpretation (Wick & Timpl, 1980). In addition, the bullous pemphigoid antigen, which is a specific basement membrane zone component of stratified epithelia, can be localized along the surface of cylindroma cell clusters (Gebhart *et al.*, 1975; Wick & Timpl, 1980).

Similar deposits of basement membrane proteins have been demonstrated in protoporphyria (Wick, Hönigsmann & Timpl, 1979), hyalinosis cutis et mucosae (Fleischmajer *et al.*, 1984) and basal cell carcinoma (Van Cauwenberge *et al.*, 1980, 1983; Weber *et al.*, 1982; Stanley *et al.*, 1982).

After staining with antibodies directed against laminin and type IV collagen the presence of focal fluorescence within the tumour parenchyma suggests that these proteins are produced by the epithelial tumour cells. This is confirmed by data obtained from the cell culture experiments, which demonstrate that a considerable proportion of the tumour cells synthesize these basement membrane components, but not the interstitial collagens, type I and type III.

From the presented data, the conclusion may be drawn that most of the 'hyaline' material of cylindroma represents an extracellular deposition of basement membrane molecules. However, these are restricted to a definitive zone encircling the tumour nests and to the drop-like deposits within the tumour islands. Staining with antibodies directed against the interstitial collagens and fibronectin shows that these proteins are also part of the tumour stroma. It is noteworthy that these experiments do not exclude the presence of other molecules, which may be included in the hyaline material. Cylindroma can therefore be classified as a basement membrane-producing tumour (Wick *et al.*, 1984). The continuous basement membrane bordering the tumour islands may act as a barrier that prevents invasive growth, and thus gives a better explanation for the relatively benign behaviour of this tumour.

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Dermal cylindromas originate from the eccrine sweat gland

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SUMMARY

Ten dermal cylindromas have been studied using histological, ultrastructural and immunocytochemical techniques. The clinical data from the ten patients have also been reviewed. The results indicate histogenesis from the intradermal coiled duct region of the eccrine sweat gland.

The dermal cylindroma is a highly characteristic lesion, the histogenesis of which remains obscure. The majority (90%) arise as small, solitary, slow-growing lesions on the head or neck. They occur with a female to male predominance of 10:1. Spectacular giant forms may occur on the scalp and are known as turban tumours. Dermal cylindromas, whilst being generally benign, may be locally invasive and malignant degeneration has been reported (Bondeson, 1978). Familial cases of multiple cylindromas occur and are inherited as an autosomal dominant trait; however the solitary form does not show any familial tendency (Crain & Helwig, 1961). Multiple cylindromas may be associated with trichoepitheliomas of the face, and cylindromas with areas showing features of trichoepithelioma or syringoma have been described (Crain & Helwig, 1961).

We have studied ten examples of cylindromas from our routine histopathology files using histological, ultrastructural and immunocytochemical techniques in an attempt to resolve the histogenesis of this tumour.

METHODS

The clinical material consisted of the most recent ten dermal cylindromas recorded in the routine SNOP index of this department. The index contains eleven cases for the period 1975–1983, these representing 0·06% of the total skin biopsies submitted during that period. On review we could not support the diagnosis in one case and this was therefore rejected. Normal skin from face, neck and axilla were used as controls.

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Histology. The tissue was fixed in neutral buffered formalin. In all cases 3–5 μm sections were cut from routine processed, paraffin wax-embedded tissue. These sections were stained with haematoxylin and eosin, PAS with and without diastase digestion, Alcian blue at pH 1·5 and 2·5, Gordon and Sweet's reticulin stain and orcein.

Immunocytochemistry. Further 3–5 μm sections were stained with peroxidase-linked polyclonal antibodies using a modified peroxidase–anti-peroxidase (PAP) technique with prior trypsinization (Mepham, Frater & Mitchell, 1979). Antibodies to fibronectin, two keratin fractions (sera 28 and 29), actin and CEA were used. Monoclonal antibodies to two molecular weight fractions of human milk fat globules (HMFG1 and HMFG2) were employed using an indirect immunoperoxidase technique. The fibronectin, CEA, PAP, swine anti-rabbit and rabbit anti-mouse immunoglobulins were obtained from Dako Immunoglobulins AG. The actin antibody was purchased from Miles-Yeda Ltd. and the HMFG1 and HMFG2 from Seward Laboratories. The keratin antibodies were prepared as described by Cotton, Kirkham & Young (1984).

Ultrastructure. In two cases unprocessed formalin-fixed material was available and this was used to prepare transmission electron micrographs on a Phillips 300 electron microscope.

RESULTS

Clinical findings. The clinical findings are summarized in Table 1.

Histology. The histological appearances are identical to the classical descriptions in the literature (Lever & Schaumburg-Lever, 1983). The tumours show a typical 'jigsaw' or 'mosaic' pattern of clumps of cells. The tumour clumps consist of large polygonal cells with clear cytoplasm. These cells contain large ovoid nuclei surrounded by a fine nuclear membrane and containing diffuse granular chromatin. There are occasional nuclei with several small basophilic nucleoli peripherally placed. The cells at the periphery of the tumour clumps are smaller, show palisading and have a basophilic oval nucleus showing some chromatin clumping. In all the tumours studied there is some lumen formation, although the degree to which this occurs is very variable. The tumours have no continuity with the epidermis. The clumps of tumour cells are

TABLE 1. Clinical data for the ten cylindroma patients

Patient	Sex	Age	Site	Duration (years)	Clinical diagnosis
I.F.	F	75	Head	2	Basal cell carcinoma
M.J.	F	72	Head	> 2	Histiocytoma
H.R.	F	66	Head	5	Basal cell carcinoma
B.S.	F	36	Head	4	Pilar cyst
W.R.	F	69	Head	4	Pilar cyst
I.B.	F	86	Head	1·5	Angioma
E.T.	F	80	Chin	< 1	Basal cell carcinoma
E.S.	F	96	Head	2·5	Squamous cell carcinoma
M.D.	F	77	Head	6 weeks	—
L.S.	F	76	Head	2	Papilloma

invested by hyaline material which stains similarly to the luminal contents with H & E and PAS stains. This PAS staining is resistant to diastase digestion, indicating a neutral polysaccharide component. The reticulin stain shows coarse fibres around the tumour clumps in the region of the eosinophilic hyaline material and finer fibres within the lumina. The luminal surface of tumour cells also stains strongly with the reticulin stain. The normal adnexae present in uninvolved skin and in control skins were also invested by dense reticulin fibres. Cells of the secretory part of the apocrine glands showed coarse granular staining of the supranuclear cytoplasm. The pattern of staining with orcein permitted further distinction between various regions of normal adnexae. Eccrine sweat gland secretory coil is surrounded by dense, orcein-positive fibres, as is the deeper part of hair follicle. The eccrine duct has only a few fine orcein-positive fibres around it, similar to the upper hair follicle and sebaceous gland. Apocrine structures have a rather coarse network of granular fibres surrounding the secretory gland and dermal duct. The staining around the cords of cells in the cylindromas resembled that of the eccrine duct, upper hair follicle and sebaceous gland. The hyaline investing material did not stain with orcein.

Immunoperoxidase staining. The immunoperoxidase technique for CEA showed striking staining of the luminal surfaces within the tumour clumps comparable to that seen on the luminal surface of the intradermal portions of the eccrine sweat duct. Neither the intraepidermal eccrine duct nor the eccrine sweat gland secretory coil showed this type of staining. Both secretory components of eccrine and apocrine glands showed weak granular staining for CEA in the cytoplasm of some cells, as did the cells lining the apocrine duct lumen.

Cylindromas stained for CEA showed the same pattern as normal sweat gland (Figs 1 and 2). The luminal surface of the cylindroma ducts also stained positively with the anti-epidermal

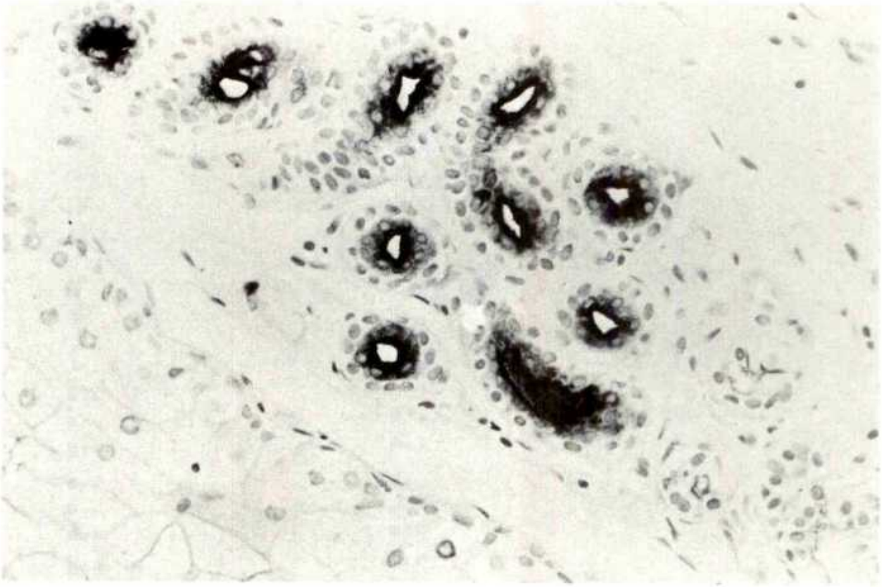

FIGURE 1. Normal sweat gland stained for CEA. Ductal staining is obvious but the secretory coil and sebaceous gland do not stain.

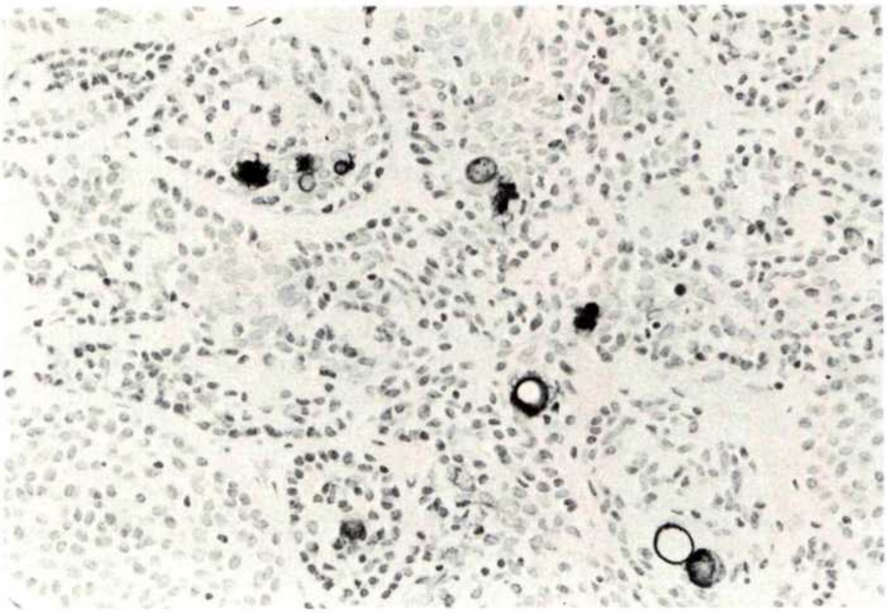

FIGURE 2. Cylindroma stained for CEA. The staining pattern is identical to that seen in sweat gland duct.

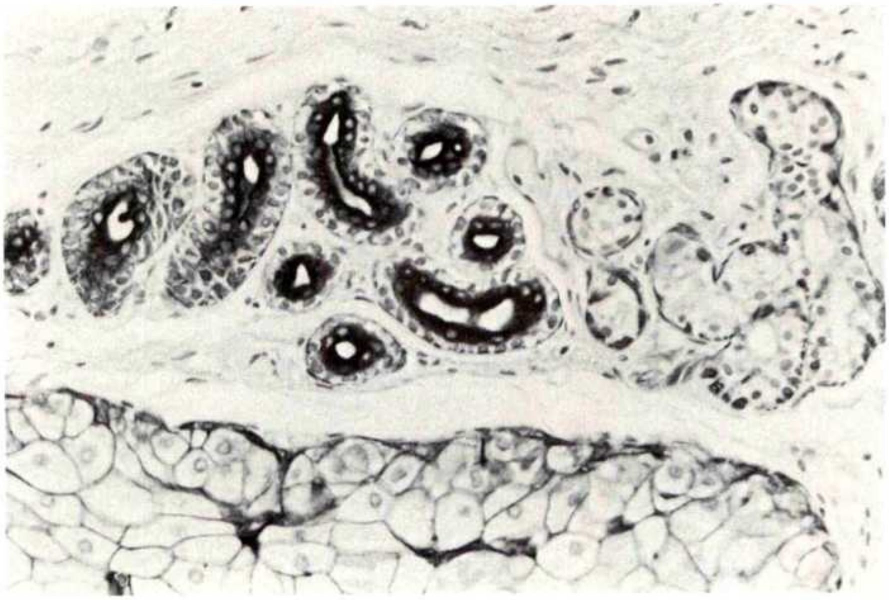

FIGURE 3. Normal sweat gland stained with serum 29. The luminal surface of the coiled duct stains strongly while the secretory coil and sebaceous gland do not stain.

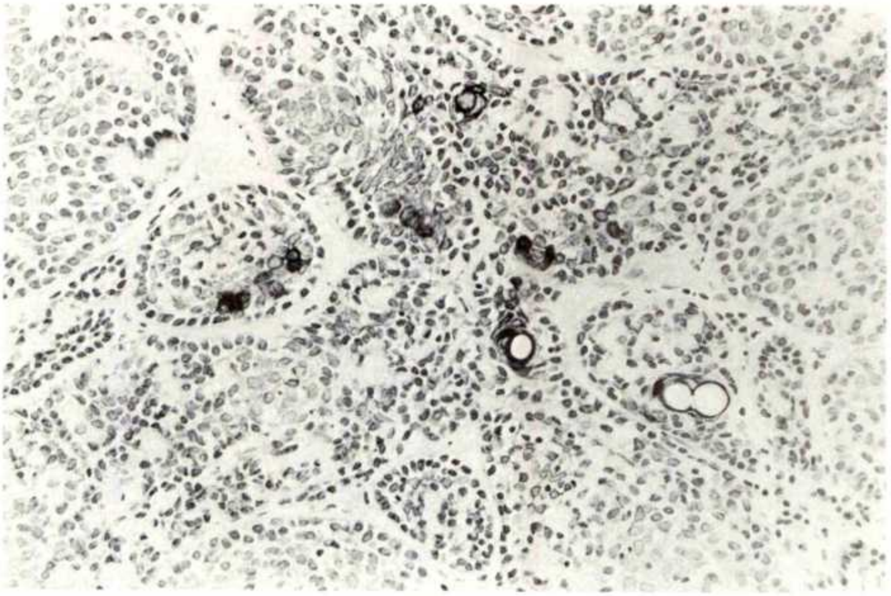

FIGURE 4. Cylindroma stained with serum 29. The staining is identical to that seen in normal coiled sweat duct.

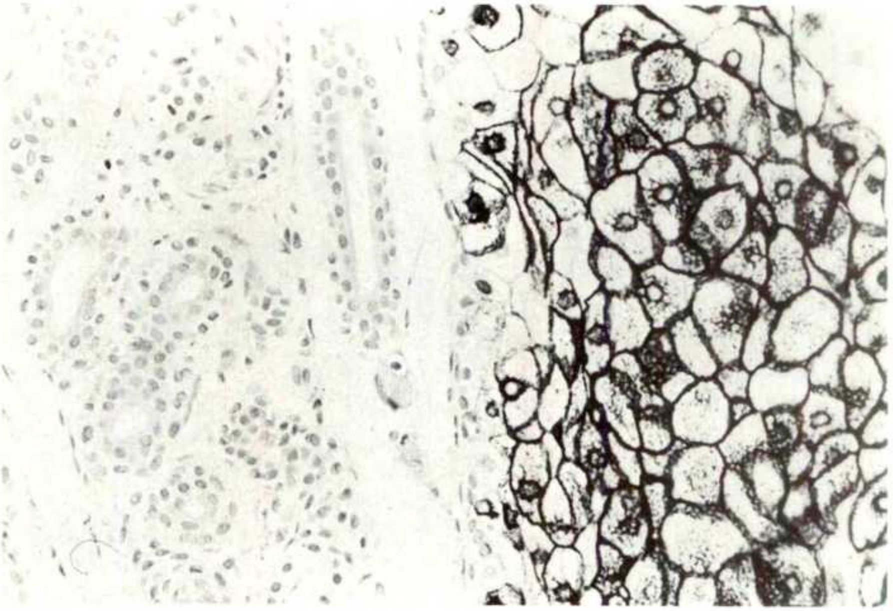

FIGURE 5. Normal sebaceous gland, sweat gland secretory coil and coiled duct stained for HMFG1. The sweat gland is negative as were the cylindromas.

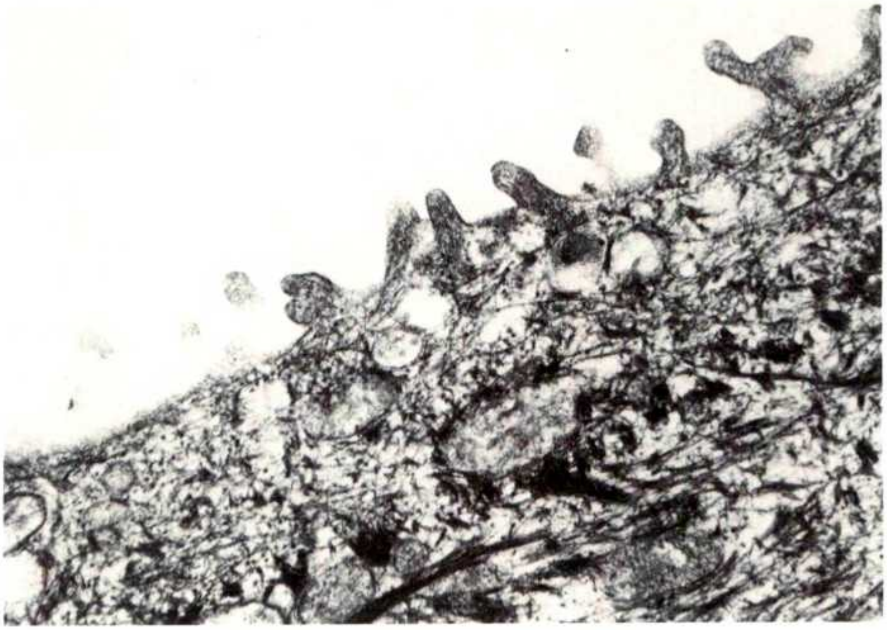

FIGURE 6. Electron micrograph of normal eccrine sweat duct showing stubby microvilli, tiny vacuoles and microfibrils. (× 27,000.)

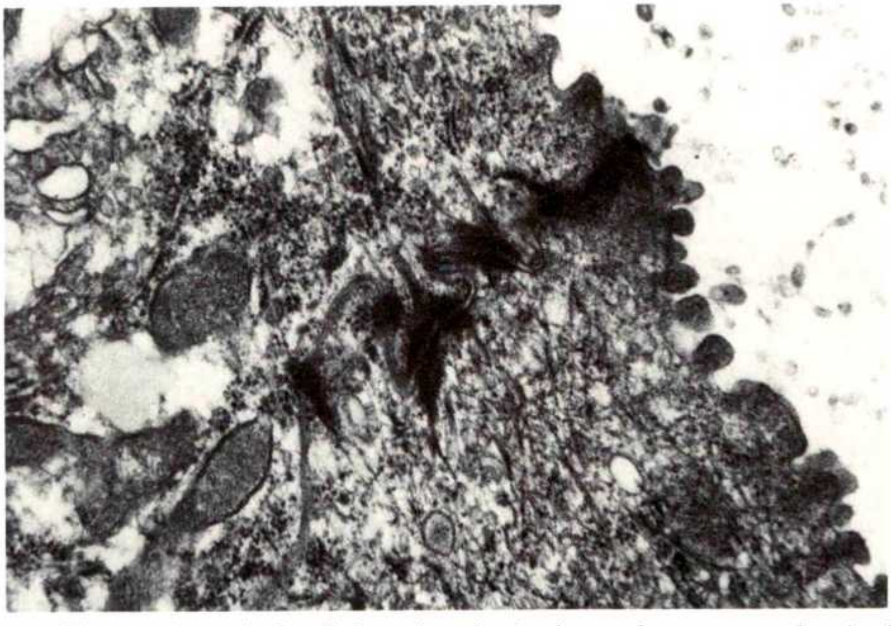

FIGURE 7. Electron micrograph of a cylindroma duct, showing the same features as normal eccrine duct and, in addition, obvious desmosomes. These features are also well described in the cells lining sweat duct lumina. (× 27,000.)

keratin serum (29). This serum stains normal epidermis, excluding the basal layer, but including that which spreads down the duct of the eccrine sweat gland and the hair follicle infundibulum (Figs 3 and 4). The luminal surfaces of cylindroma ducts also stained with serum 28, an anti-keratin antibody that differentially stains basal epidermis, rather than the upper epidermal layers.

Myoepithelial cells in both eccrine and apocrine sweat glands could be demonstrated by actin staining. No such staining occurred in any areas of the cylindromas. HMFG1 and HMFG2 stained sebaceous glands. Figure 5 shows HMFG1 staining in sebaceous gland with a negative sweat gland next to it. HMFG2 stained these structures more strongly than HMFG1, although the distribution of both antigens appeared to be similar. HMFG1 did not appear to stain eccrine duct or gland, unlike HMFG2 which stained eccrine gland with a weak, thin, linear surface pattern and eccrine duct in a similar, but stronger, fashion. Apocrine glands showed a granular surface staining with HMFG1 and 2. HMFG1 and 2 did not stain cells in the tumour clumps. Occasional luminal surfaces of cylindroma ducts stained with HMFG2, but not with HMFG1.

Fibronectin stained the hyaline material surrounding the tumour masses but not the hyaline luminal contents. It did not stain the tumour clumps.

Electron microscopy. The ultrastructure of a normal eccrine duct is shown in Fig. 6, with a cylindroma duct wall at the same magnification in Fig. 7. The similarities between cylindroma and eccrine duct are obvious. Intradermal straight duct and coiled duct cannot be distinguished with confidence on electron microscopy alone.

DISCUSSION

Although most authors would admit that the 'dermal cylindroma' is a specific entity, with a characteristic morphology, there is still much debate concerning its histogenesis. Various reports have suggested apocrine gland (Lever & Schaumburg-Lever, 1983; Milne, 1972; Torsuev *et al.*, 1977), eccrine gland (Crain & Helwig, 1961; Kwitken, 1981) and hair follicle (Mehregan & Rahbari, 1978) as origins. These reports have attempted to specify the substructure or cell of origin of this tumour. Hashimoto and Lever (1969) believe that these tumours simulate intradermal apocrine duct structure while Crain and Helwig (1961) believe that the tumours are derived from eccrine dermal duct, Kwitken (1981) advances a myoepithelial cell origin from eccrine gland analagen of the secretory segment and Mehregan and Rahbari (1978) have suggested derivation from, or differentiation towards, apocrine gland and hair follicle.

Light microscopy (Kwitken, 1981) and electron microscopy (Lever & Schaumburg-Lever, 1983; Crain & Helwig, 1961) have been used in the investigation of this tumour.

Immunohistochemical staining for CEA has been reported by Penneys, Nadji & Morales (1982) but this technique has not previously been used to investigate the histogenesis of these tumours. We have used all the above methods in a study of a group of ten cylindromas.

We believe that the results support the view that these tumours are of eccrine origin and more specifically that they are derived from the coiled segment of the intradermal duct between straight duct and secretory coil. The staining characteristics of the investing hyaline material suggests an origin from secretory coil or coiled intradermal eccrine duct but not the straight portion of the intradermal eccrine or apocrine duct or from apocrine gland itself, none of which have this investing material as prominently developed as areas of the eccrine gland and duct. This material is absent from around hair follicle structures.

The reticulin staining pattern of cylindromas supports an origin from eccrine coiled duct or secretory coil as distinct from comparable apocrine structures. The absence of reticulin staining in the cytoplasm of the large pale cells of cylindromas also argues against an apocrine gland origin for these cells.

The possibility of a myoepithelial origin for the tumour can be rejected on the basis of the immunohistochemical reactions for actin. This antibody stains myoepithelial cells in normal dermal adnexae but there was no comparable staining in any of the cylindromas. The distinction between secretory cell and myoepithelial cell actin staining was very marked. The anti-keratin antibodies reacted with the cuticle of eccrine and apocrine ducts. The cuticle lining the lumen of cylindroma ducts also stained with the anti-keratin antibodies. The exact nature of the cuticular lining of tumour and eccrine ducts remains obscure, but the two linings do seem to be identical.

Staining for CEA was strong on the surface of all areas of the eccrine duct, as well as weakly granular in some areas of eccrine secretory cell cytoplasm. Apocrine secretory cells showed weak cytoplasmic staining and apocrine ducts were also only weakly positive for CEA. The cylindromas showed very strong luminal staining whilst tumour cells in solid areas did not react. Thus the appearance of this luminal antigen seems to be very specific in both eccrine duct and tumour duct formation.

HMFG1 could not be demonstrated in, or on, cylindroma cells within the solid or duct areas. Apocrine gland and duct stained strongly for this material. HMFG2 showed no staining of cylindroma cells in the solid areas of the tumour but there was strong staining of the tumour lumen. This staining pattern contrasts with the granular staining of apocrine gland. Eccrine gland and duct shows the same differential staining pattern with HMFG1 and HMFG2 as seen in cylindromas. Ultrastructurally, in those central areas of the solid tumour clumps where cell adhesion was breaking down, presumably the first stages of duct formation, the appearances were identical with those seen in the embryonic development of eccrine duct structures (Hashimoto, Gross & Lever, 1966). The formed ducts themselves were also very similar to mature eccrine intradermal straight duct and coiled duct, showing short villi, microfibrils and sparse vacuoles. These appearances contrast strongly with those of secretory coil and we cannot therefore support the view of Reynes *et al.* (1976) who suggest an origin from this structure.

Our cylindromas showed neither electron microscopic nor immunohistochemical appearances that would support derivation from myoepithelial cells of either apocrine or eccrine origin.

The completely intradermal situation of the tumour, its curious mosaic growth pattern, the presence of the investing hyaline material, the formation of lumina in some tumour cell clumps, the occasional presence of normal eccrine secretory gland in the adipose tissue deep to the tumour and our above-reported immunohistochemical results lead us to propose that the cylindroma is derived from the coiled portion of the intradermal eccrine duct.

ACKNOWLEDGMENTS

We should like to gratefully acknowledge the skilled technical help of Mr Brian Mepham and the staff of the Experimental Pathology Department.

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Immunoperoxidase anti-keratin staining of epidermal and pilar cysts

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A Charm Against Wens

Wen, wen, little wen
Here you must not build, nor have any dwelling,
But you must go North from here to the hill close by
Where, poor wretch, you have a brother.
He will lay a leaf at your head.
Under the foot of the wolf, under the wing of the eagle
Under the eagle's claw, may you grow ever weaker.
Wither like coal at the hearth,
Shrink like muck in the wall
And evaporate like water in the bucket.
Become as small as a grain of linseed
And much smaller than a hand-worm's† hip-bone,
And become so small that you become
Nothing at all.

Translated from the Anglo-Saxon by Linda Cotton.
Royal MS 4A. xiv, British Museum.*

SUMMARY

Epidermal and pilar cysts have been stained with anti-keratin antibodies raised from human hair and human callus. The staining properties of the two cysts are different and, in general, support the idea that they originate from epidermis and trichilemmal hair follicle layer respectively.

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* In: *The Anglo-Saxon Minor Poems* (Ed. by E. van Kirk Dobie) (1942). George Routledge and Sons, London.
† Hand-worm = scabies mite.

Correspondence: Dr D.W.K.Cotton.

The distinction between epidermal and pilar cysts is now well established (Leppard, 1980), as is their non-sebaceous origin. Clinically they have long been lumped together as 'wens' or 'sebaceous cysts', but Kligman's famous paper on 'The Myth of the Sebaceous Cyst' (Kligman, 1964), although not the first, was the final blow to the concept. Subsequently opinion seems to have settled to the idea that epidermal cysts are derived from normal epidermis, including that which extends down the infundibulum of hair follicles and sweat glands, and that pilar cysts are derived from the trichilemmal layer of the hair follicle. This accounts for the alternative name for pilar cysts—trichilemmal cysts.

These tumours are usually of little importance. Some are known to undergo malignant transformation but this is a rarity (Saida *et al.*, 1983). Multiple epidermal cysts are a marker for Gardner's syndrome (Leppard, 1980). This is a diagnosis worth making, for without treatment 100% of these subjects may be expected to develop carcinoma of the colon. However, these structures are of interest in their own right since they pose, and consequently may help to resolve, many of the basic problems of the histogenesis of tumours.

We have therefore studied a number of epidermal and pilar cysts with immunoperoxidase stains using two anti-keratin antibodies.

METHODS

Twenty cysts, ten pilar and ten epidermal, were obtained from the SNOP index of routine biopsies at Southampton General Hospital. They had all been fixed in 10% neutral buffered formalin, processed into paraffin wax and sectioned at 3–5 μm within 2 days of initial fixation and

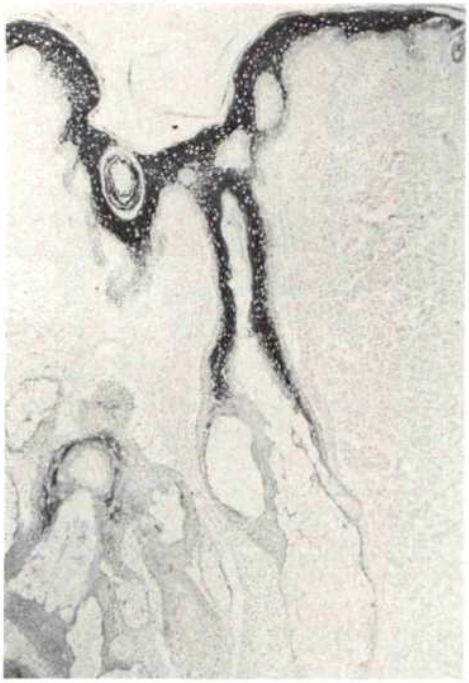

FIGURE 1. Normal epidermis and hair follicle stained with serum 29.

stained routinely with haematoxylin and eosin. Further sections were cut at 3–5 μm and these were used for the immunoperoxidase staining.

Keratin antibodies were raised to human hair clippings (i.e. containing no hair root material) and to pooled callus and nail flakes.

Hair keratin was extracted in 8 M urea/0·2 M potassium thioglycollate at pH 10·8, carboxylated and precipitated against 0·8 M acetate buffer at pH 4·4. This was followed by exhaustive dialysis against tap water (Gillespie & Inglis, 1965).

Epidermal keratin was obtained by alkaline extraction and selective isoelectric precipitation at pH 5·5. These preparations were then used to raise antibodies in rabbits as described by Bauer (1972).

The serum raised against hair keratin extract is subsequently referred to as serum 28 and that raised against callus extract as serum 29. The antibodies were used as primary antisera in a PAP staining system with prior trypsinization as described by Mepham *et al.* (1979).

RESULTS

Figure 1 shows normal epidermis and hair follicle stained with serum 29. There is marked staining above the basal layer and extending through the granular layer. The hair follicle shows a similar pattern of staining in the infundibulum but the deeper areas, including the trichilemmal layer, do not stain. The mature keratin of the epidermis stains strongly.

Figure 2 shows a hair follicle stained with serum 28. There is no staining of the follicle itself but the hair above the bulb stains strongly. The trichilemmal layer does not stain at all.

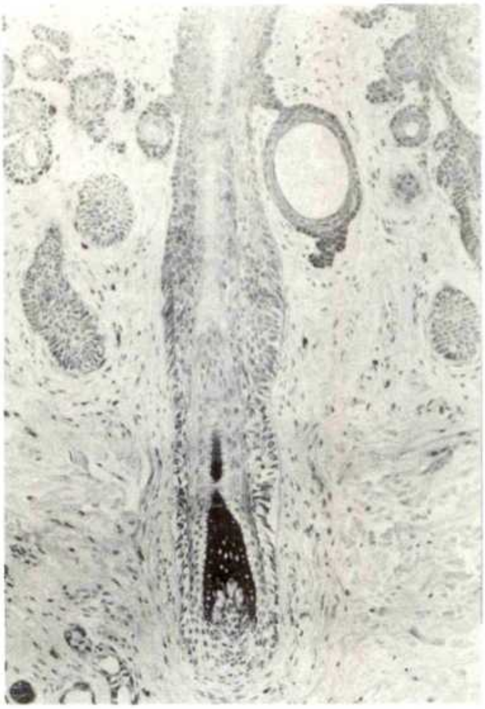

FIGURE 2. Hair follicle stained with serum 28.

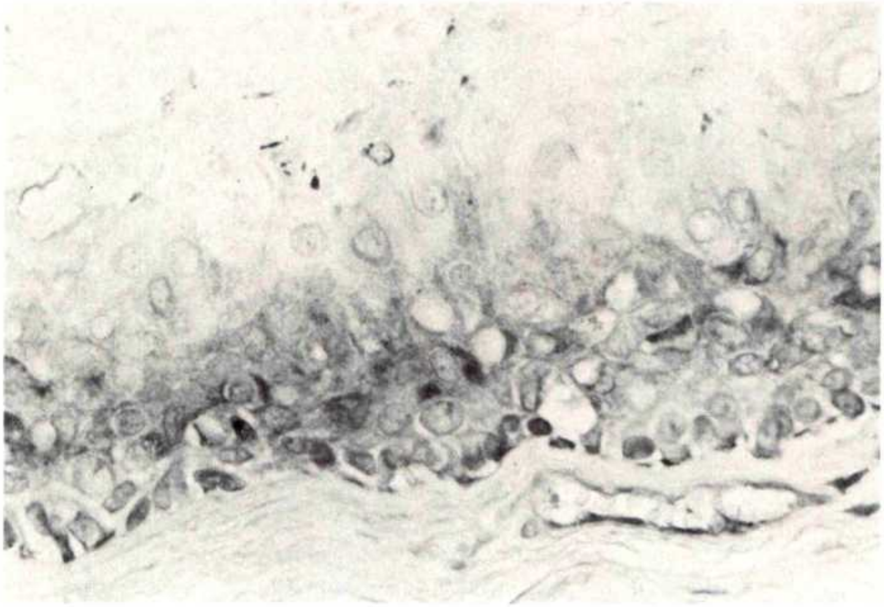

FIGURE 3. Pilar cyst stained with serum 28.

Figure 3 shows a pilar cyst wall stained with serum 28. All cell layers stain to some extent but the basal layers stain most strongly while the contents do not stain at all.

Figure 4 shows an epidermal cyst stained with serum 29. In this case the epidermal cyst wall stains very strongly above the basal layer. The contents of the cyst also stain strongly.

These results are summarized in Table 1.

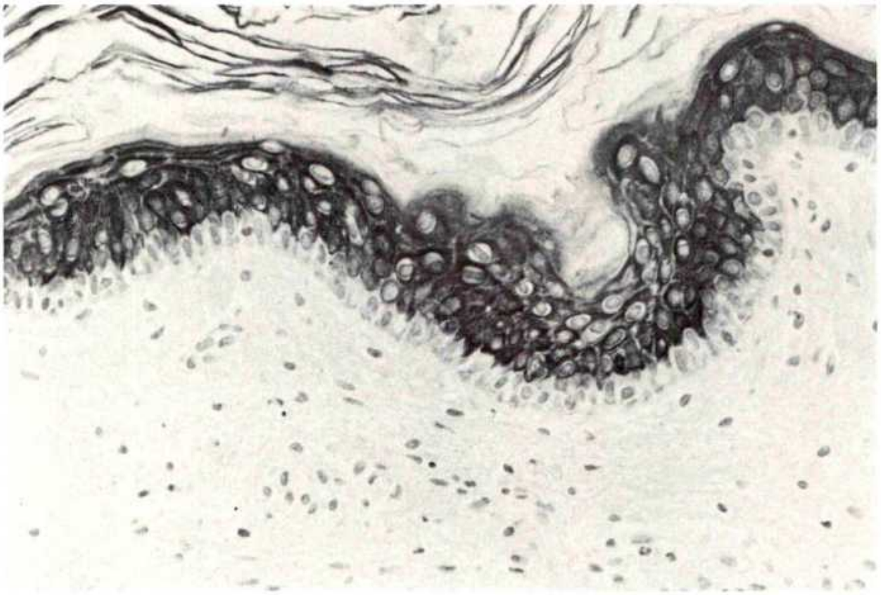

FIGURE 4. Epidermal cyst stained with serum 29.

TABLE 1. Pattern of staining with IP anti-keratins in epidermal and pilar cysts

	Hair antibody (28)	Callus antibody (29)
Epidermal	Weak lining	Strong lining
	Weak contents	Strong contents
Pilar	Strong lining	Weak lining
	Weak contents	Weak contents

DISCUSSION

The current view is that epidermal cysts are derived from epidermis while trichilemmal cysts are derived from the trichilemmal layer of the hair follicle. The arguments for these origins are mainly morphological and cytological (Pinkus, 1969). The lining of epidermal cysts is structurally indistinguishable from normal epidermis in its mode of keratinization, which occurs via the production of a pronounced granular layer with keratohyalin granules. The main distinction from normal epidermis is that the cyst wall never supports adnexae. The material contained within epidermal cysts has all the usual histological characteristics of lamellar keratin. Pilar cyst walls also lack adnexae. The mode of keratinization in this case, if that is what is happening, is very similar to that seen in the trichilemmal layer of the hair follicle. Histologically, no keratohyalin granules are seen, although with the electron microscope trichohyalin granules can be identified (Lever & Schaumberg-Lever, 1983). The trichilemmal layer of the hair follicle is continuous with the epidermis above the layer of the infundibulum and its character changes at about that level.

Using two anti-keratin antibodies derived from two different keratin fractions, we have shown that the antigenic properties of pilar and epidermal cysts are different, as would be expected from their morphology. The epidermal cysts stain exactly as though they were normal epidermis. The pilar cysts stain differently from the epidermal cysts, but not in the same way as the trichilemmal layer of the hair follicle. The contents of the pilar cyst do not stain with either serum but hair stains with both sera. This would seem to suggest that our sera have some degree of cross-reactivity, as might be expected considering their origins, but that none of the contained antibodies is specific for the so-called 'keratin' of the trichilemmal layer of the hair follicle. The mode of production of this keratin is different from epidermal keratin, and it is not believed to contribute to the final keratinized product, in this case hair. Certainly, nothing that we have extracted from mature keratins shows antigenic activity with any of the layers of the root sheath, but our extracts do not contain any hair root material.

An incidental finding was that the luminal surface of eccrine sweat ducts stained strongly with serum 29 whilst the sweat gland coil showed no staining with either sera. This would also lend support to the idea that those minute epidermal cysts that appear in the skin following blisters are derived from sweat ducts (Tsuji, Sugai & Suzuki, 1975).

It would seem therefore that epidermal cysts are derived from epidermis, including those regions of epidermis that extend down the upper sweat duct and down to the infundibulum of the hair follicle, but that the keratin produced is the typical epidermal product and distinct from hair keratins. On the other hand, pilar cysts show more similarities to, but not antigenic identity with, the trichilemmal layer of the hair follicle, as suggested by Pinkus (1969).

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Replication of Paget cells in mammary and extramammary Paget's disease

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SUMMARY

Replication of Paget cells in mammary and extramammary Paget's disease was evaluated by autoradiography after incorporation of tritiated thymidine. Neoplastic cells proliferated in the deepest layers of the epidermis suggesting a topographical control of their multiplication similar to that of the normal epidermis.

Neoplastic cells of mammary and extramammary Paget's disease share some common histological features. They have a large nucleus with a prominent nucleolus and abundant pale cytoplasm. They are distributed singly or in groups of various sizes and shapes throughout the epidermis and adnexal epithelium. These distinctive histological features have been referred to as Paget cells distributed in a pagetoid pattern (Jones, Austin & Ackerman, 1979).

We have studied the rate of proliferation of Paget cells, and evaluated the relationship between the replication of Paget cells and epidermal cells.

METHODS

We used standard optical autoradiography after incorporation of tritiated thymidine, in two cases of mammary Paget's disease and one case of extramammary Paget's disease.

The first patient aged 39 years had a 1-year history of Paget's disease of the areolar region of the breast, associated with a carcinoma of the lactiferous ducts. The second patient aged 68 years suffered for more than 3 years from a mammary apocrine carcinoma with Paget's disease of the breast. The third patient aged 82 years had a mastectomy in 1970 for a breast carcinoma associated with mammary Paget's disease. Three years later an erythematous oozing macule appeared on the vulva that progressively enlarged and became nodular. After 7 years of evolution a biopsy proved the diagnosis of extramammary Paget's disease associated with an underlying adenocarcinoma restricted to the dermis.

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Ten μCi of tritiated thymidine ([^{3}H]TdR, sp. act. 27 Ci mmol/l, Institut des Radioéléments, Fleurus, Belgium) was incorporated in a flash labelling of 1 hour. Step sections were done through entire specimens and processed for conventional autoradiography using Kodak NTB2 emulsion and an exposure time of 28 days. They were stained with hemalum-eosin-saffron or with PAS and colloidal iron.

Labelled keratoblasts and labelled Paget cells were distinguished according to their location and the size of the nucleus and cytoplasm. Mitotic figures and [^{3}H]TdR-labelled cells were counted in the epidermis. Results were expressed as number of labelled cells per mm^2 of stratum

TABLE 1. Labelling and mitotic indices of Paget cells and keratoblasts in the epidermis of mammary and extramammary Paget's disease

Type of Paget's disease	Paget cells		Keratoblasts	Paget cells
	Mitosis (%)	[^{3}H]TdR LI	[^{3}H]TdR LI	+ keratoblasts N[^{3}H]TdR/mm^2
Mammary	1·3	19	18	8400
Mammary	2·2	26	13	12,300
Extramammary	<0·5	9	12·5	4000

N[^{3}H]TdR/mm^2 represents the total number of labelled Paget cells and keratoblasts per mm^2 of surface of the stratum corneum

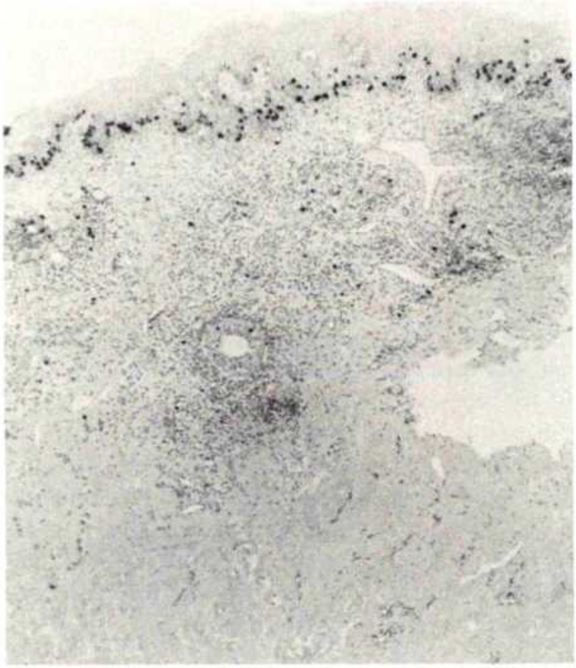

FIGURE 1. [^{3}H]TdR autoradiography of mammary Paget's disease. Labelled cells are frequent in the deep layers of the epidermis and in the lactiferous duct.

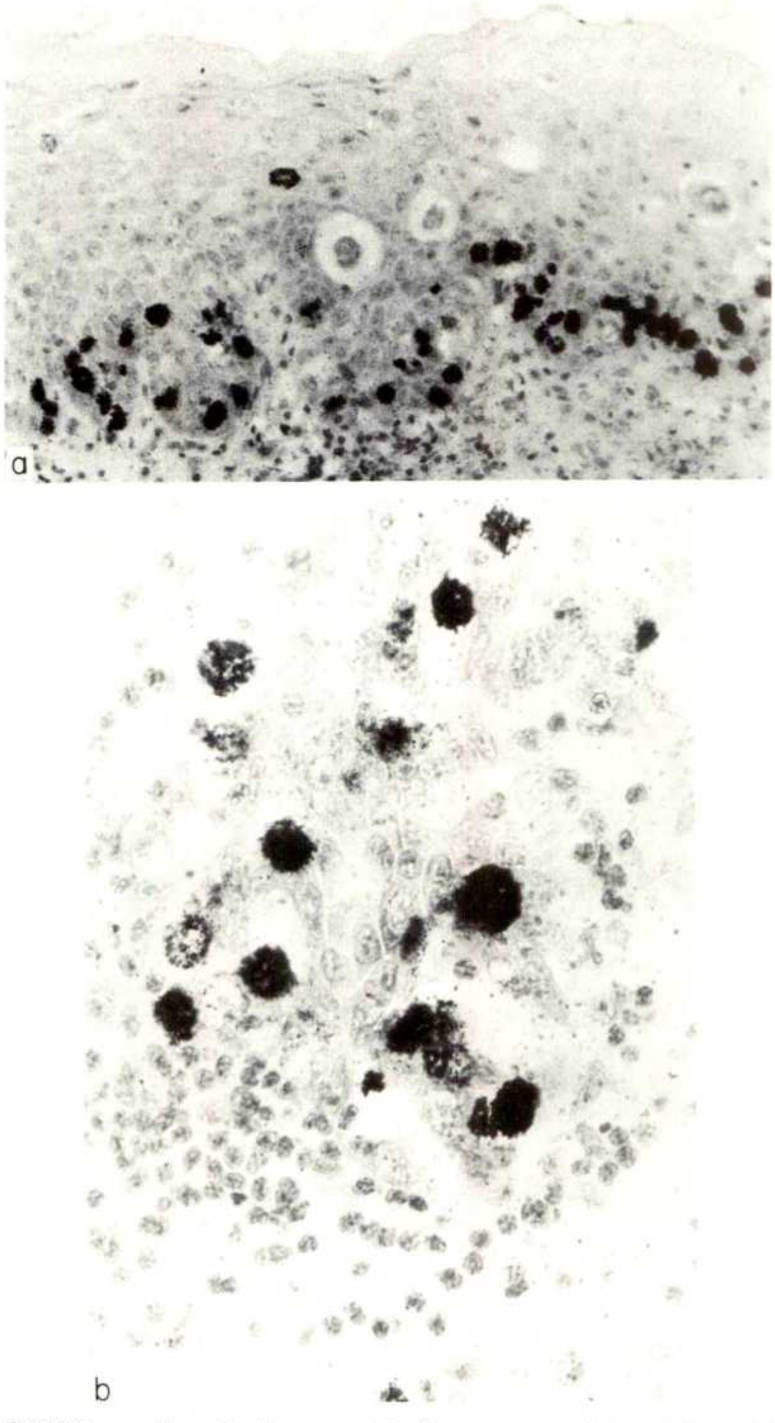

FIGURE 2. [^{3}H]TdR autoradiography of mammary (a) and extramammary (b) Paget's disease. Many small nuclei of keratoblasts and large nuclei of Paget cells are labelled in the lower mid-part of the epidermis.

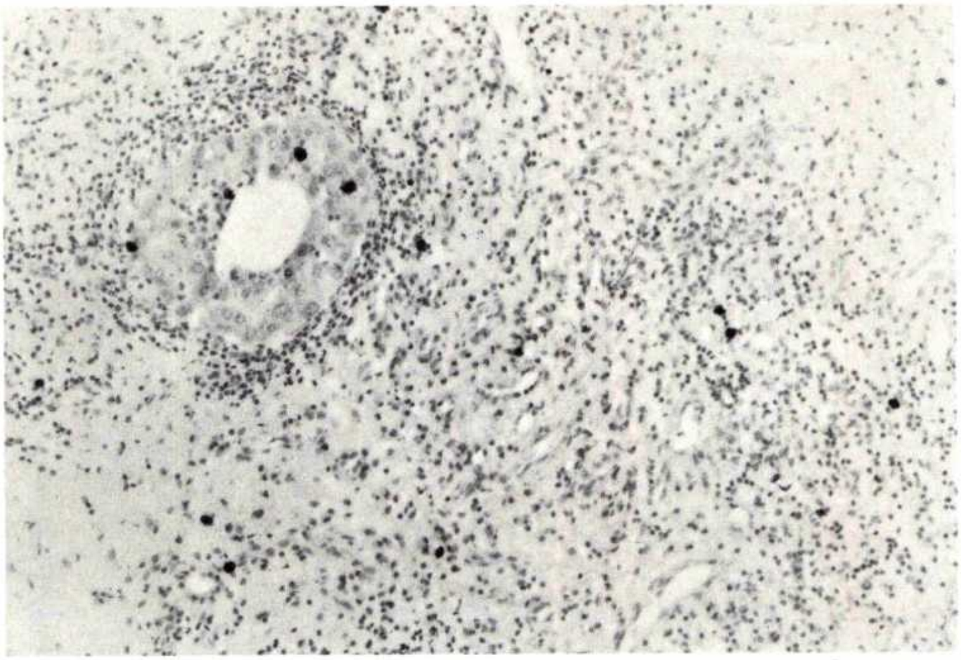

FIGURE 3. [^{3}H]TdR autoradiography of mammary Paget's disease. Neoplastic cells in the lactiferous duct as well as some fibroblasts and endothelial cells are labelled.

corneum (normal value 25 ± 7 keratoblasts/mm^2) and as a labelling index, estimating the percentage of labelled Paget cells among the total number of Paget cells. A dermal labelling index representing the number of labelled dermal cells per mm^2 of dermis (normal value lower than 5/mm^2) was also evaluated.

RESULTS

In mammary and extramammary Paget's diseases [^{3}H]TdR-labelled Paget's cells were almost exclusively confined to the lower part of the epidermis, in the region where the proliferative pool of epidermal cells normally occurs (Figs 1 and 2). In the uppermost layers of the epidermis, the Paget cells were usually not synthesizing DNA except when the epidermis was almost

TABLE 2. Labelled cells in the dermis of mammary and extramammary Paget's diseases

Type of Paget's disease	Labelled endothelial cells per mm^2 of dermis	Other labelled dermal cells per mm^2 of dermis
Mammary	5	12
Mammary	1	1
Extramammary	12	2

completely replaced by the neoplasm. Paget cells grouped in nests were frequently labelled in mammary Paget's disease, but at a much lower extent in our patient with extramammary Paget's disease. In lactiferous ducts, Paget cells displayed the same intensity of labelling as in the epidermis (Fig. 3).

Pertinent data concerning the epidermal involvement in mammary and extramammary Paget's diseases are presented in Table 1. Within the dermis, the figures strikingly differ from one patient to another (Table 2).

DISCUSSION

There is little information in the literature concerning the rate of replication of Paget cells in mammary and extramammary Paget's diseases (Mitchell, 1974). In both of them we observed a striking topographical relationship between [^{3}H]TdR-labelled Paget cells and labelled keratoblasts. This could be due to a common biological control of proliferation for these two cellular pools and to parallel processes of maturation of cells evolving towards the surface of the epidermis. Changes in the structure of epidermal cells adjacent to Paget cells (Sagebiel, 1969; Medicina & Sahihi, 1972) could be related to our observations.

The presence of many Paget cells inside the normal germinative compartment of the epidermis is associated with a four- to five-fold increase in the number of keratoblasts synthesizing DNA. This observation recalls the increase in the number of labelled keratoblasts we have observed when the epidermal germinative pool is dissociated either by inflammatory cells (Pierard, Pierard-Franchimont & de la Brassinne, 1983) or by an acantholytic process (Pierard-Franchimont & Pierard, 1983). It could be related to some biological feature resembling a loss of contact inhibition promoting cellular growth. The increased number of epidermal cells synthesizing DNA and the high number of replicating Paget cells in the S and M phase of proliferation lead to an estimated 100- to 500-fold increase in the total number of proliferating cells per mm^2 of stratum corneum. This is not accompanied by a parallel increase in volume of the tissue, and therefore the rates of cellular migration and loss through the surface of the epidermis are probably large.

The wide range of proliferative rates observed in the dermis is difficult to interpret. It could be due to differences in dermal structure between the areola and genital skin, to differences in the intensity of the inflammatory reaction or to distinct growth factors released by the neoplasm.

We conclude that mammary and extramammary Paget's disease should be regarded as neoplasms characterized by a large pool of germinative cells whose proliferation is in part topographically controlled within the epidermis. At the site of infiltration by the neoplastic cells, the epidermal cells display a high rate of proliferation.

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Recovery from porphyria cutanea tarda with no specific therapy other than avoidance of hepatic toxins

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SUMMARY

Little is known of the natural progression of untreated porphyria cutanea tarda. We report sixteen cases (fourteen sporadic and two familial) in which the cutaneous and biochemical abnormalities improved without any specific therapy other than the avoidance of hepatic toxins.

Porphyria cutanea tarda (PCT), the commonest porphyria (Mayer & Schmid, 1978), is characterized by cutaneous fragility, high levels of porphyrinuria, and damage to the liver. Little is known of the spontaneous course of the untreated disease, but there are isolated descriptions of remission after stopping ingestion of drugs or alcohol (Lundval, 1982; Enriquez de Salamanca *et al.*, 1982; Haberman, Rosenberg & Manon, 1975).

Ippen (1982) divided patients with PCT into two prognostic groups: (1) non-treated patients, who progress towards cirrhosis, with a decrease in porphyrin production and subsequent death; (2) patients treated with repeated phlebotomy and/or chloroquine, who have a favourable prognosis, with clinical recovery and normalization of the urinary porphyrin pattern within 4 to 6 months. In the treated hereditary forms, even after 10 to 12 years the urinary porphyrin pattern is still pathological (Ippen, 1982).

Our experience with hundreds of cases has allowed us to observe the disappearance of the skin changes in many patients and the return of the urinary porphyrin excretion to normal values. This mainly occurs in subjects with progression to hepatic cirrhosis and subjects receiving chloroquine therapy or repeated phlebotomy, but in this report, we describe a third group, namely patients who spontaneously recover after the exclusion of liver toxins.

Other than the avoidance of hepatic toxins, the only therapy to which these patients were submitted, was that of the so-called 'hepato-protectors' (Silymarin, folinic acid etc.). This is our usual regime with all new patients, who are kept under observation for 1 year (with checks every 3 months). After this period, if we observe an improvement in the cutaneous and biochemical changes, the observation period is prolonged. The patients who show no improvement are treated with specific therapy such as phlebotomy or chloroquine.

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The sixteen patients presented here were from a series of 320 patients examined during the past 14 years. We have not given the percentage of spontaneously recovered patients because some of the 320 cases have been lost to follow-up.

CASE REPORTS

All of the following cases (two with known inheritance; Topi & D'Alessandro Gandolfo, 1977) presented with specific cutaneous manifestations, a high porphyrinuria and porphyrinaemia, a typical urinary pattern for PCT (over 70 to 80% of uro- and heptacarboxylic porphyrins) and impaired liver function.

In all cases the following investigations were performed: urinary delta-amino-levulinic acid, aspartate amino transferase (GOT), alanine amino transferase (GPT), glutamate dehydrogenase (GLDH), sorbitol dehydrogenase (SDH), cholinesterase (CHE), γ-glutamyl transferase (γ-GT), alkaline phosphatase (AP), oral glucose tolerance test, tolbutamide test, protein electrophoresis, fractionated serum bilirubin, iron and copper in the serum, total iron binding capacity, prothrombin activity, erythrocyte sedimentation rate, full blood count, BSP retention, immunoglobulins, α_1-fetoprotein and Australia antigen. All the cases were submitted to hepatic scintigraphy and eight cases to hepatic biopsy.

The urinary and serum porphyrins were determined by the methods of D'Alessandro Gandolfo and Topi (1973, 1976). The main details are shown in Tables 1 to 3.

Case 1. He stopped drinking alcohol, his skin rapidly improved and the altered tests became normal. Scintigraphic examination was normal.

Case 2. After having stopped his intake of drugs and alcohol, his skin became normal within 2 years and the hyperporphyrinuria disappeared within 3 years. All the tests are now normal. The scintigraphic examination shows only a very slight irregularity of the radiocolloid distribution.

Case 3. The patient stopped his intake of alcohol, the skin slowly improved and there was a slow decrease of porphyrinuria. After 2 years, acute hepatitis developed with jaundice, acholic faeces, an increase of GOT and GPT (without inversion), bilirubin, γ-GT and alkaline phosphatase, but the serum amylase was normal. The jaundice then regressed and all the tests which were initially altered became normal.

Case 4. Red cell urodecarboxylase was equal to 67% of the normal values, at the first visit. He stopped drinking but refused liver biopsy and hepatic scintigraphy. After 2 years the cutaneous manifestations disappeared and, after 4 years, the hyperporphyrinuria and the transaminase returned to normal. Eight years later all the tests were normal. This patient is a member of a family in which three cases of PCT have been found (Topi *et al.*, 1977) and is the brother of case 5. Another brother with porphyria and cirrhosis, died of hemorrhage from oesophageal varices at the age of 36.

Case 5. During admission to hospital for hepatitis, at the age of 27 years, he presented the typical cutaneous signs of PCT. Red cell urodecarboxylase was 58% of the normal values. The scintigraphic examination showed an extremely irregular distribution of the radiocolloid. After 1 year the skin changes disappeared, and after 5 years the hyperporphyrinuria also disappeared.

TABLE 1. Clinical features of sixteen PCT patients

Case	Sex	Age (years)	Disease duration	Follow-up (years)	Alcohol intake (ml/day)	Hepatotoxic substances	Other diseases	Liver biopsy
1	M	34	6 months	7	168	—	—	—
2	M	34	3 months	10	150	Sulpha-drugs Non-steroidal anti-inflammatory agents	—	CPH *
3	M	54	4 years	5	120	—	Hepatitis	CPH *
4	M	44	4 years	9	130	—	—	—
5	M	30	3 years	11	130	Isoniazid	Tuberculosis Hepatitis (AU$^+$)	CPH *
6	M	60	5 months	10	190	Herbicides Pesticides Isoniazid	Tuberculosis Prostatic cancer	CPH *
7	F	66	3 months	7	120	Hypoglycaemic & anti-parasitic agents	Diabetes	CPH *
8	M	60	5 years	7	180	Diazepam	Hepatitis Gastritis Pleuritis	CPH *
9	F	39	1 year	6	45	Norgestyl & oestradiol	Microcythemia Hepatitis	—
10	M	62	6 months	8	130	Indomethacin Ketoprofen	Urethritis Malaria	CPH *
11	M	41	1 year	11	102	Testosterone	—	—
12	F	11	4 months	7	24	Aminopyrine	—	CPH *
13	M	55	1 year	5	170	—	Duodenal ulcer Renal lithiasis Atrial fibrillation	—
14	F	60	5 years	4	45	—	—	—
15	M	41	1 year	3	80	—	—	—
16	M	46	2 years	13	70	—	—	—

* Chronic persistent hepatitis.

Case 6. The patient stopped drinking alcohol and avoided contact with anti-parasitic agents and herbicides. After 6 months the cutaneous signs disappeared, and after 2 years the hyperporphyrinuria and the hepatomegaly had disappeared. After 3 years all the tests were normal. Four years later the patient was submitted to surgery for carcinoma of the prostate, followed by oestrogen therapy. Notwithstanding this therapy, which lasted 6 years, there was no increase in urinary phorphyrins and no skin changes, but there was a slight increase in the transaminases. After another 4 years, bone metastases occurred and the patient died 3 years later.

Case 7. At scintigraphic examination, a diffuse abnormality of the radiocolloid distribution was evident. Glucose tolerance test showed a diabetic curve. The patient ceased drinking wine, no longer took hypoglycaemic drugs and avoided contact with anti-parasitic agents. The cutaneous manifestations disappeared after 2 years and urinary porphyrins became normal after 6 years. All the tests, except the glucose tolerance test, are now normal.

TABLE 2. Biochemical features at presentation

Case	GOT* (mU/ml)	GPT* mU/ml)	γ-GT* mU/ml)	CHE (mU/ml)	AP (mU/ml)	GLDH (mU/ml)	SDH (mU/ml)	BSP (%)	Total bilirubin (mg%)	Direct bilirubin (mg%)	Prothrombin activity (%)
1	18	17	65	4000	18	5	0·7	6	2·3	0·5	93
2	70	56	68	3556	40	5	4·0	13	1·0	0·2	71
3	23	35	25	4015	15	4	2·0	6	0·8	0·4	85
4	34	37	280	2377	38	2	—	9	1·1	0·1	100
5	32	53	27	3627	40	2	1·8	12	0·9	0·3	50
6	142	114	39	3580	41	8	5·6	23	1·3	0·6	—
7	33	27	81	3056	38	2	0·7	13	0·9	0·3	100
8	49	38	560	2293	29	7	3·3	29	2·7	1·0	87
9	26	20	22	2246	7	7	1·0	7	0·8	0·2	76
10	25	27	57	3557	17	8	1·2	6	0·7	0·2	93
11	31	48	45	2527	33	4	2·1	14	0·7	0·4	60
12	26	36	15	6177	76†	2	0·5	12	1·1	0·3	108
13	89	76	91	1853	22	21	8·1	21	0·5	0·2	81
14	23	49	23	869	73	10	1·9	4	0·6	0·1	100
15	23	37	76	2808	20	4	1·9	7	0·8	0·2	82
16	32	35	11	2878	30	2	7·1	4	0·6	0·2	—
Normal values:											
Male	0–12	0–12	6–28	1200–3800	12–30	0–4	0·4	5	1·0	0·2	70–120
Female	0–12	0–12	4–18	1200–3800	12–30	0–3	0·4	5	1·0	0·2	70–120

* See text for key to all abbreviations.

† Normal values for children: 24–86 mU/ml.

TABLE 3. Serum and urinary porphyrins

Case	Serum porphyrins (mg/l) before	Serum porphyrins (mg/l) after	Urinary porphyrins (mg/24 h) before	Urinary porphyrins (mg/24 h) after	Pattern of urinary porphyrins (after) % Uro	7-COOH	6-COOH	5-COOH	Copro
1	0·08	0·01	6·04	0·13	29	10	2	3	56
2	0·04	n.d.	6·36	0·06	23	11	3	2	61
3	0·06	n.d.	2·01	0·04	24	5	5	3	63
4	0·07	0·01	7·94	0·13	14	8	3	4	71
5	0·04	n.d.	5·79	0·09	21	5	4	4	66
6	—	0·01	7·34	0·07	—	—	—	—	—
7	0·06	0·01	2·14	0·08	38	10	3	5	44
8	0·14	0·01	3·24	0·14	27	14	5	4	50
9	0·16	n.d.	8·25	0·09	37	7	16	5	35
10	0·08	n.d.	2·45	0·07	22	5	2	2	69
11	0·22	0·01	6·48	0·07	41	17	3	5	34
12	0·19	n.d.	2·69	0·06	7	1	3	6	83
13	0·13	n.d.	3·32	0·11	26	3	1	13	57
14	0·03	n.d.	1·63	0·13	17	4	2	2	75
15	0·10	0·01	5·01	0·08	28	6	3	4	59
16	—	n.d.	1·21	0·05	20	5	1	3	71
Normal values:									
	≤0·01	≤0·01	≤0·15	≤0·15					

Case 8. The scintigraphy showed a finely irregular distribution of the radiocolloid. On stopping ingestion of alcohol and diazepam, the cutaneous lesions were cured within 5 months, and the increase of the urinary porphyrins disappeared in 2½ years.

There are now only very slight increases of GOT, GPT, SDH, IgG, IgA and direct bilirubin levels. The BSP retention dropped to 11%. The scintigraphic examination is normal.

Case 9. On scintigraphy, a diffuse abnormality of the radiocolloid distribution was revealed. She refused to be submitted to hepatic biopsy. She reduced her alcohol intake to a modest quantity and stopped her oral contraceptive drugs. After 1 year the cutaneous signs disappeared and after 4 to 5 years the urinary porphyrins became normal. All of the tests are now normal.

Case 10. The scintigraphy showed a diffuse abnormality. The patient stopped drinking and 2 years later, notwithstanding his occupation (bricklayer), the cutaneous fragility disappeared and after 5 years the hyperporphyrinuria had disappeared. All the tests are now normal, except for a very slight residual increase of IgG and transaminases and a slight abnormality on scintigraphy.

Case 11. On stopping ingestion of alcohol and androgens, the cutaneous signs disappeared and after 7 years the porphyrinuria was normal. Today all the tests are within normal values.

Case 12. This case was recently presented elsewhere (Topi *et al.*, 1983). No other family member had ever been affected. Red cell urodecarboxylase was equal to 70% of the normal value. On stopping the very modest intake of ethanol and aminopyrine (taken for headaches), the cutaneous signs disappeared within 2 years and the scintigraphy, as well as the other tests, returned to normal.

Case 13. A diffuse irregularity of the radiocolloid distribution and modest splenic increased uptake was found during the scintigraphic examination. On stopping alcohol, the skin changes disappeared within 2 years, and after 3 years the urinary and serum porphyrins became normal. The BSP retention dropped to 12% and only a slight increase of the gammaglobulins remained. The scintigraphic examination has remained unchanged.

Case 14. The scintigraphy was within normal limits. After 1 year the skin signs disappeared and after 2½ years the urinary porphyrins became normal. All the tests are now normal.

Case 15. The scintigraphy revealed hypoactive areas, towards the centre of the liver. The cutaneous changes returned to normal within 1 year of stopping alcohol and, after 2½ years, the excretion of urinary porphyrins and the other laboratory tests were normal.

Case 16. On stopping alcohol, the skin improved within 1 year, and there was a reduction of the urinary porphyrins within 3 years. After 9 years, during which he had received no therapy, the patient returned for review. The urinary porphyrins were normal, as was the hepatic scintigraphy. Only the transaminases were slightly elevated.

DISCUSSION

These cases, which are unusual compared with others reported in the literature, pose several questions.

The first is 'Can these patients be considered cured?' Certainly the diagnosis of porphyria cutanea tarda can no longer be made on clinical or biochemical criteria.

The second question is 'What specifically has, and what has not, recovered in these patients?' The answer is not clear, because in some patients (1, 2, 4, 5, 6, 7, 11, 14, 15) all of the tests presenting evidence of a hepatic involvement became normal, but in others, although improvement occurred, some abnormalities persisted. Similarly the urinary porphyrin excretion usually became normal, but in others, some abnormality persisted. The porphyrins in the serum always became normal.

The third question is 'What are the common characteristics of these cases?' The only common characteristics are (a) the histological diagnosis of persistent chronic hepatitis without siderosis, a form that is susceptible to spontaneous recovery even with a variable quantity of fibrosis; and (b) the avoidance of potentially hepatotoxic substances.

These observations, however, provoke a series of questions concerning the basic enzymatic defect in PCT.

It is known that a reduction of urodecarboxylase is found in PCT (Kushner, Barbuto & Lee, 1978; van Tieperman *et al.*, 1978a,b; Felsher, Norris & Shih, 1978; Elder *et al.*, 1980) and quantitation of this enzyme (unfortunately carried out in only three of our cases, all showing modest decreases) might permit hereditary forms of PCT to be distinguished from the sporadic ones (de Verneuil, Aitken & Nordmann, 1978). In the former the deficit would be more striking, although in both cases, external toxins would be required to produce clinical manifestations of PCT.

From our survey this does not appear to be so, since both familial and sporadic cases responded equally well to removal of hepatic toxins. Contrary to what Ippen (1982) observed, in our familial cases (cases 2 and 5) the urinary porphyrins returned to the normal pattern (71 and 66% of coproporphyrins, respectively).

Iron does not appear to have been an important pathogenetic factor in our cases. In fact, only four of our cases showed a modest hypersideraemia (less than 200 μg %) and in none of the liver biopsies performed was there hepatic siderosis.

Emphasizing the importance of exogenous factors for provoking clinical PCT, we may mention another patient we observed, who had all the cutaneous signs of PCT and a porphyrinuria of 5·89 mg/24 h. After giving up his heavy drinking, the clinical signs improved within a few months and in 1½ years the urinary porphyrins had decreased to 0·25 mg/24 h. At another medical centre he later received treatment with testosterone and methyltestosterone, even without drinking again, and after 9 months he again presented the typical cutaneous changes of PCT, together with an increase of urinary porphyrins to 4·56 mg/24 h.

We therefore consider the metabolic defect in PCT to be potentially reversible, depending on exposure to exogenous hepatic toxins.

To elucidate fully the relationship between the enzymatic defect and exogenous pathogenetic factors, serial monitoring of all the enzymes of the biosynthetic chain of the haem (not limited only to the urodecarboxylase), would probably be necessary in various tissues (liver, fibroblasts in culture, erythrocytes), with a prolonged patient follow-up. For the time being, this is not within our capability.

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The assessment of acne vulgaris—the Leeds technique

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SUMMARY

We describe two simple, reproducible scoring systems for assessing acne severity, and we emphasize the technical problems which could invalidate either technique. Constant baseline data is desirable for any clinical trial, and our data clearly show that acne patients should ideally be off all treatment for at least 2 months before the start of a therapeutic trial.

There are many ways of assessing therapy in acne vulgaris, but few authors have published the reproducibility and accuracy of their clinical trial methods (James & Tisserand, 1958; Witoski & Simons, 1966; Frank, 1971; Pillsbury, 1971; Michaelson, Juhlin & Vahlquist, 1977; Cook, Centner & Michaels, 1979; Allen & Smith, 1982). We describe in detail the two methods which we have found to be the most useful and statistically reproducible.

The first, an overall assessment of the acne severity in a particular area (grade between 0 to 10), is accurate, reproducible, rapid and suitable for use in the routine clinic. The second, for detailed work in therapeutic trials, uses a counting system in which we distinguish between active and less active acne lesions. This technique is relatively simple and reproducible when carried out with the attention to detail we recommend.

METHODS

1. The grading technique

The two authors graded a total of 666 patients, but not all patients were examined by both doctors. Tables 1 and 2 indicate how many patients were seen by each doctor on the same occasion and how many patients were seen by the same doctor on a different occasion. In the latter case the reassessment of the patient was made within 24–48 hours of the first. All patients were assessed under a Brighton 1001 circular fluorescent lamp situated within 30 cm of the patient. The room was also well illuminated with ceiling fluorescent lighting. The physician assessed the patient by looking at and feeling the skin. The grading took only 30–60 seconds, and the grading scale was 0 (no acne whatsoever) to 10 (the most severe acne). We subdivided groups 0 to 2 into seven further subgroups: 0·25, 0·5, 0·75, 1·0, 1·25, 1·5 and 1·75. Grades 0·25 to 1

TABLE 1. The intradoctor assessment. The number of observations relate to those that agree or fall within 0·5–2·0 of a grade from the initial observation

Difference between consecutive gradings	Number of observations: Face A	Face B	Back A	Back B	Chest A	Chest B
0	210	230	91	96	89	93
±0·5	74	103	80	79	82	89
±1·0	12	12	5	11	4	4
±1·5	10	11	4	4	6	5
±2·0	0	0	1	1	0	0
Total	306	356	181	191	181	191
r	0·92	0·94	0·89	0·89	0·81	0·94

TABLE 2. The interdoctor assessment. The number of observations relate to those that agree or fall within 0·5–2·0 of a grade from the initial observation

Difference between doctors' grading	Number of observations: Face	Back	Chest
0	49	42	39
±0·5	46	39	42
±1·0	4	3	3
±1·5	3	2	3
±2·0	0	1	0
Total	102	87	87
r	0·89	0·87	0·80

represent patients with physiological acne or 'acne minor', whereas those with grades of 1·5 or more have clinical acne, or 'acne major' (Kligman, 1974). In this group (1·5 to 10) the acne was further subdivided into 0·5 divisions. Forty per cent of the subjects had physiological acne, 60% suffered from clinical acne.

Three sites were graded: face, back and chest. The face included the chin and neck anterior to the sternomastoid muscles. The chest in men was from the waist upwards, but in women only the skin between the breasts and above the bra was included. The back was the area from the waist upwards, in both sexes. Shoulders were included for both the back and chest assessment, but the arms were excluded.

Most observations were of the face but the backs and chests of a smaller number of patients were also graded (Table 1). Figures 1 and 2 show examples of acne from grades 0·5 to 8 on the face, but it must be emphasized that black and white photography does not accurately reflect the clinical assessment.

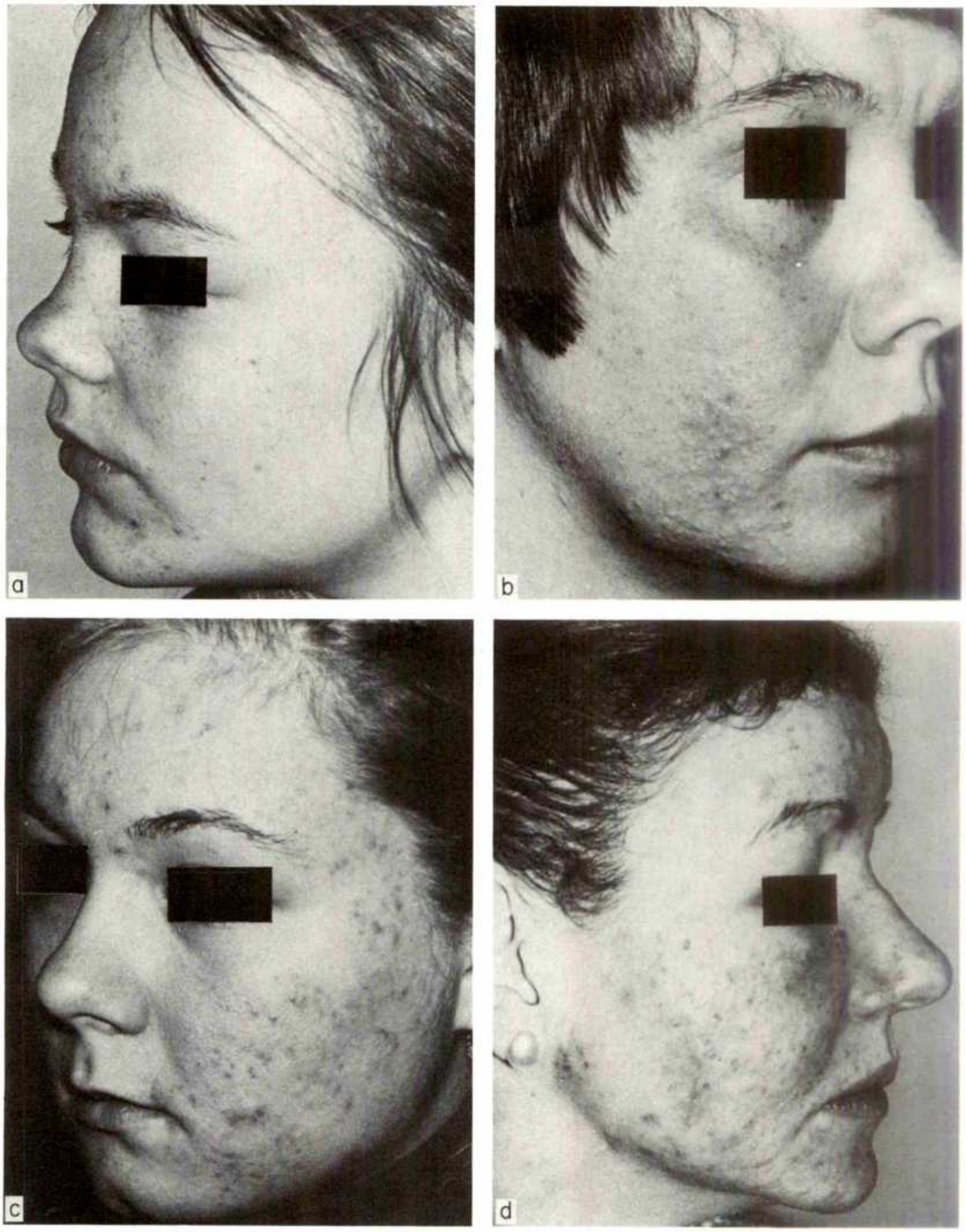

FIGURE 1. Shows four grades of acne and these are: (a) 0·5, (b) 0·75, (c) 1·0, (d) 1·5. Note that the patient with grade 1·5 appears to have a similar grade to the one in the lower left hand corner (1·0). This emphasizes the need for palpation. The patient in the bottom right hand corner (1·5) had more palpable lesions.

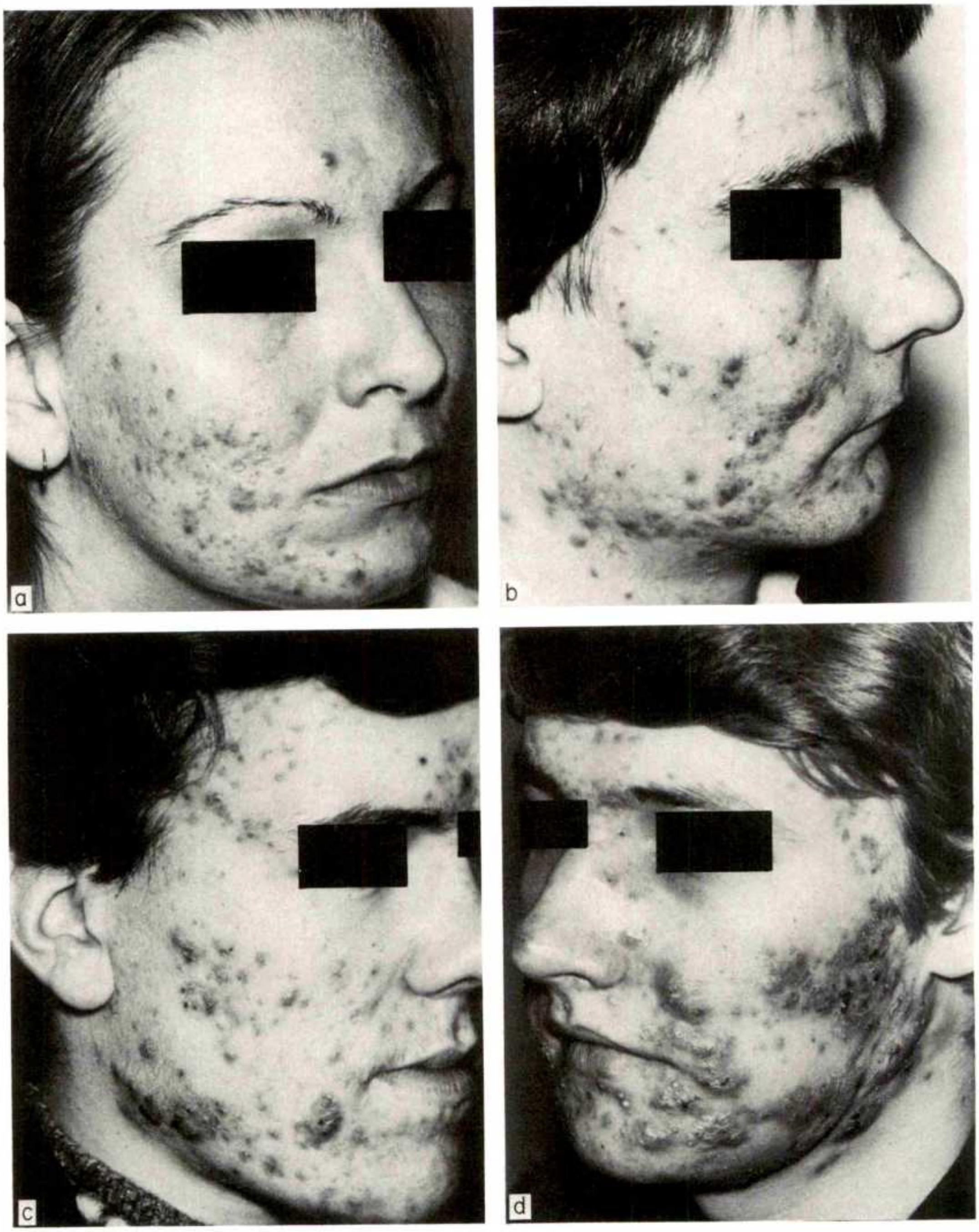

FIGURE 2. Shows four further grades which are as follows: (a) 2·0, (b) 3·0, (c) 5·0, (d) 8·0.

Before starting this study, we had a training period in order to improve our scoring ability. There must be some graduated relationship between the individual scores, but the precise relationship between the grades is unlikely to be strictly linear and so we used the non-parametric analytical techniques of Wilcoxon and the Mann–Whitney U test. Intra- and interdoctor comparison was also made by determining Pearson's coefficient of correlation. Further support for this statistical approach is supported by the fact that the distribution of acne grades within the population investigated was not normal.

2. The counting technique

Lesions were divided into inflamed and non-inflamed, as follows.

(a) *Non-inflamed lesions* were blackheads and whiteheads. Any intermediate lesions were counted according to their major component. Prominent follicles, small milia or trichostasis spinulosa must be rigorously excluded as they occur frequently and would badly skew the results.

(b) *Inflamed lesions* were either superficial (papules and pustules) or deep (nodules, cysts and deep pustules).

(i) *Superficial papules and pustules* varied in size from 0·1 cm (with minimal erythema) to 0·5 cm (with a marked macular flare). The smaller less inflammatory lesions were referred to as 'less active papules or pustules', the larger erythematous lesions as 'active papules or pustules'. Some 40% of lesions fell between these two types but in practice we assigned the lesion according to its major component.

(ii) *Deep inflamed lesions* were predominantly nodules which were 0·5 cm or larger. Palpation was essential, since some nodules were almost invisible but easily palpable.

(iii) *Macules* represented the resolving phase of either superficial or deep lesions and were either large or small. They should be included in acne assessment as they contribute to the overall degree of inflammation. As they evolve they become brown and much less distinct. The counting of individual lesions may then be very difficult.

In order to confirm the reproducibility of the method, two doctors (A and B) examined 432 patients. Many patients were counted on consecutive days in order to compare reproducibility by the same doctor and a comparison between doctors was made assessing the same patients on the same day.

Statistical analysis was by the paired t-test and Pearson's coefficient of correlation.

Lesion counting is not easy and perfection takes time. Attention to the following points will help to avoid errors.

(1) The patient should be sitting comfortably so that the observer can move around him easily to count each area.

(2) In addition to good background fluorescent lighting, we recommend a Brighton 1001 fluorescent lamp, which can be easily moved to illuminate both sides of the patient during the examination.

(3) When counting, we divide the face into the right and left sides and count both sides. In some patients the lesions are clustered around the midline, making a right–left division difficult; we then count the forehead, the cheeks and chin separately and combine the counts. Some authors count lesions from one area only. As acne affects several sites and may improve at one site as it deteriorates at another, we prefer to count the whole face.

(4) Palpation is necessary because some macules may look like a nodule, but on palpation show no depth at all. Conversely, a nodule may hardly be visible and yet can be felt to lie deep in the skin.

(5) Stretching of the skin will increase the number of whiteheads and blackheads that are visible, but as the degree of stretching might vary, this is not permitted. For similar reasons, we recommend that no lens is used. If a lesion is impalpable, and not obvious with good lighting, then such a vague lesion is best ignored.

Pitfalls for the unwary

Prominent follicles. Confusion of non-inflamed lesions with prominent follicles is a problem around the nose and on the chin, especially in mid-teenagers. In therapeutic trials, we recommend that non-inflamed lesions are not counted either on the nose or around the edge of the nose.

Sandpaper acne. Two per cent of youngsters have the so-called 'sandpaper' acne. In these patients the forehead is covered with many (usually 100 or more) very superficial lesions, which are impossible to classify correctly. Such patients should be excluded from clinical trials.

Hair styles. Many young people change their hair style frequently. Long, uncut hair may mask non-inflamed lesions, and we avoid counting them around the hair line. However, there is usually no difficulty in recognizing inflamed lesions in this area.

Shaving. Patients may grow a moustache or a beard during the trial and this will complicate the results. Patients may also develop low grade folliculitis on the chin and neck as a result of shaving trauma. However, the papules and pustules associated with a folliculitis are much less easily felt than acne lesions. Patients should shave daily, preferably at a constant time, as stubble can affect the interpretation of all lesions.

Cosmetics. Despite advice to the contrary, some females will use make-up. This must be removed and the patient observed 30 minutes later, when the erythema resulting from washing has settled.

Ultraviolet radiation. This will camouflage the non-inflamed lesions and make the inflamed lesions look less inflamed. For this reason, trials should not be performed in the summer.

TABLE 3. The intradoctor comparison for the major types of lesions. The data are expressed as percentages

Difference between counts expressed as a percentage of the original count	Type of lesion					
	Total non-inflamed	Blackhead	Whitehead	Total superficial inflamed	Papules	Pustules
0	8	6	5	9	11	9
0–5	58	62	59	61	65	56
5–10	11	7	11	12	14	10
15–20	12	11	11	10	5	15
20–25	7	10	8	6	4	7
25+	4	4	6	2	1	3
r	0·87	0·86	0·85	0·92	0·94	0·89
P	$<0·001$	$<0·001$	$<0·001$	$<0·001$	$<0·001$	$<0·001$

Other dermatoses. Sycosis barbae may occur in association with acne and low grade seborrhoeic eczema may simulate a primary irritant dermatitis seen commonly with such treatments as benzoyl peroxide.

3. Establishing a baseline for clinical trials

When performing clinical trials in acne our subjects have not used acne treatment for at least 4 weeks before the start of the study. We have now tried to define how quickly acne lesions relapse after stopping therapy. We investigated twenty-two subjects who had been using 5% benzoyl peroxide gel in a clinical trial of 3 months' duration. They agreed not to treat their skin for 2 months so that we could assess the rate of return of their lesions. All patients were seen at monthly intervals by the same doctor and assessed using our grading and counting techniques.

RESULTS

Grading

Table 1 shows that the correlation for the same doctor grading at different times was good, especially for the face and back. The correlations between two doctors (Table 2) were numerically lower but still good. The Mann–Whitney U test also showed no significant difference between either doctor at any site.

Counting

Tables 3 and 4 show the intra- and interdoctor comparison for the major lesion types. Both sets of data showed good correlation. The comparison between two doctors was slightly inferior to the assessment made by the same doctor, but there were no significant differences. Assessment of non-inflamed lesions was less accurate than was the assessment of inflamed lesions. The occasional individual result was undoubtedly quite different, but as a group, the overall comparisons were most acceptable.

TABLE 4. The interdoctor comparison for the major types of lesions. The data are expressed as percentages

Difference between counts expressed as a percentage of the original count	Type of lesion					
	Total non-inflamed	Blackhead	Whitehead	Total superficial inflamed	Papules	Pustules
0	6	5	4	7	9	7
0–5	55	57	55	59	65	50
5–10	13	9	11	11	14	16
15–20	13	13	13	12	4	15
20–25	8	11	10	8	6	8
25+	5	5	7	3	2	4
r	0·83	0·82	0·81	0·86	0·91	0·82
P	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001

Establishing a baseline for clinical trials

The results indicate quite clearly that grade and lesions significantly increase up to the eighth week after stopping therapy (Table 5).

TABLE 5. The acne grade and lesion counts (mean ± s.e.m.) at T3, which represents the third month of treatment with benzoyl peroxide. T3 also represents the day at which treatment stopped. S1 and S2 refer to the observations made at one and two months after stopping this treatment

	Visit	Mean ± s.e.m.	Statistical comparison of either T3/S1 or T3/S2
Grade	T3	0·18 ± 0·02	—
	S1	0·32 ± 0·04	0·01
	S2	0·37 ± 0·05	0·01
Blackheads	T3	0·76 ± 0·30	—
	S1	2·42 ± 0·68	0·005
	S2	4·47 ± 1·14	0·001
Whiteheads	T3	11·66 ± 2·21	—
	S1	22·00 ± 3·90	0·001
	S2	30·50 ± 4·86	0·001
Total non-inflamed lesions	T3	12·57 ± 2·36	—
	S1	24·42 ± 4·12	0·001
	S2	34·83 ± 4·83	0·001
Papules—active	T3	1·71 ± 0·44	—
	S1	3·47 ± 0·90	0·05
	S2	4·72 ± 0·66	0·01
Papules—less active	T3	7·23 ± 0·83	—
	S1	12·90 ± 1·88	0·001
	S2	15·05 ± 2·18	0·001
Total papules	T3	8·95 ± 1·18	—
	S1	15·90 ± 2·63	0·005
	S2	19·77 ± 2·45	0·001
Small pustules—active	T3	0·19 ± 0·11	—
	S1	0·42 ± 0·14	NS
	S2	0·44 ± 0·21	NS
Small pustules—Less active	T3	0·42 ± 0·16	—
	S1	1·09 ± 0·31	NS
	S2	1·15 ± 0·30	0·05
Total small pustules	T3	0·61 ± 0·25	—
	S1	1·61 ± 0·46	0·05
	S2	1·42 ± 0·42	0·05
Total small inflamed lesions	T3	10·33 ± 1·57	—
	S1	17·85 ± 2·84	0·005
	S2	21·88 ± 2·78	0·001
Macules	T3	4·61 ± 0·65	—
	S1	4·66 ± 0·88	NS
	S2	2·89 ± 0·47	0·025

NS = not significant.

DISCUSSION

Our techniques of grading and counting are not perfect, but they are reproducible. They have taken several years to develop and in each study we recognized some technical error in our assessment. For the past 18 months we have not been able to improve the technique, nor have we been able to highlight any further pitfalls.

It is difficult to compare our technique precisely with other techniques, since most have been reported as part of a clinical trial and such data often lacks evidence of reproducibility (Witkowski & Simons, 1966; Michaelsson *et al.*, 1977). Some authors do attempt to define the size of their lesions and even give a different score to each lesion type (Witkowski & Simons, 1966; Michaelsson *et al.*, 1977), a small non-inflamed lesion being scored much less than a nodule for example, but this can be criticized on the grounds that we do not know really the precise ranking value number of individual lesions. Absolute counts are parametric data but scores ascribed to lesions are non-parametric. It is probably wrong to mix these two types of data.

Some investigators have only counted certain parts of the face but as the whole of the face may develop acne, and we do not know whether there is a mosaic pattern in its development, we prefer to count the entire face.

We know of no previous techniques in which there is an attempt to count active and less active lesions. We think this is important, as the initial effect of a drug may be to hasten the resolution of inflamed lesions. We initially tried to classify superficial inflamed lesions as either developing or resolving but in 30% of cases we were proven wrong. We also are convinced of the value of including macules, particularly since some patients have up to thirty. Macules contribute to the assessment of the overall grade. Treatment with active anti-acne agents, such as benzoyl peroxide, initially increases the number of macules (Schutte, Cunliffe & Forster, 1981). Perhaps this is because these agents shorten the natural history of inflamed papules and the ratio of macules to papules increases. Eventually, such therapy reduces the macule count.

Photography has three major drawbacks (Cook *et al.*, 1979). First, there are technical problems. Constant lighting, a constant distance between the patient and camera and constant developing procedures are essential and difficult to maintain accurately. Second, photographs will never adequately detect the small non-inflamed lesions. Third, photography is two-dimensional; it can never replace palpation, and errors in distinguishing deep lesions from active superficial lesions or macules will occur.

It has also been suggested that an overall grading system can replace spot counting (Allen & Smith, 1982). There is no doubt that our grading system is quick and reproducible and eminently suitable for use in the clinic, but we doubt the wisdom of discarding spot counting in therapeutic trials. We have shown to our own satisfaction on several occasions that spot counting is a more sensitive technique in that it distinguishes small differences better than an overall grade. A grade assessment is also complicated by the presence of erythema and scaling—a not infrequent side-effect of several effective topical treatments. Spot counting also gives some insight into the morphogenesis of acne lesions before and during treatment.

The secret of lesion counting is practice. Before embarking on a clinical study, the assessors' reproducibility should be checked. We suggest that the novice assessor selects 30 patients and, in groups of 10, counts the lesions (under standard conditions) on one day and then 24 hours later. The more spot counting performed, the more spots are 'seen' until a plateau is reached. When this plateau is achieved, then comparison of data collected on two consecutive days should give a correlation with a minimum value of 0·80. Only at this stage is the physician ready to count spots in a clinical trial.

The grading system requires less practice and it does not matter whether one doctor's grading is identical with another, so long as the individual is consistent.

Problems may arise in allocating a grade to patients with numerous non-inflamed and few inflamed lesions or those with few very isolated deep inflamed lesions. Both inter- and intradoctor correlation tends to be poor in these cases and we usually acknowledge this by marking the notes 'difficult to grade'. Lesion counting usually represents no problems in these patients.

The grading technique is useful for routine clinical assessment, but also for clinical investigators who are relating accurately measured variables like sebum excretion rate and bacterial counts to acne.

In an ideal world subjects should probably be off all treatment for 3 months before starting a study but in practice this is normally impossible. However, this study highlights the importance of ensuring that subjects are off treatment for a stated minimum period before a clinical trial in order to establish an adequate baseline, particularly when comparing drugs whose efficacies may be similar, nor should cross-over studies in acne be performed without a minimum 3-month washout period.

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Pharmacology and Treatment

Methotrexate in lymphomatoid papulosis

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SUMMARY

The effect of weekly methotrexate treatment (5 to 25 mg orally) was studied in three patients with lymphomatoid papulosis. In all three patients a good clinical effect was obtained. Skin biopsies revealed no sign of lymphomatoid papulosis after treatment. Methotrexate appears to be effective in controlling lymphomatoid papulosis and is possibly superior to PUVA in this respect.

Lymphomatoid papulosis, first described by Macaulay in 1968, is a continuing self-healing eruption, clinically benign, but histologically malignant. The disease has been considered to have a benign course in most cases, but in about 10% malignant lymphoma develops (Weinman & Ackerman, 1981). Some authors think it to be a distinct entity although with a broad spectrum of different clinical types, while others have proposed that lymphomatoid papulosis could be subdivided into 'classical' benign lymphomatoid papulosis, lymphomatoid papulosis associated with parapsoriasis en plaque or mycosis fungoides, and finally as a primary cutaneous T-cell lymphoma (Lange Wantzin, Thomsen & Hou-Jensen, 1984). PUVA treatment has been shown to control this disease (Lange Wantzin & Thomsen, 1982). We have now treated three patients with methotrexate (MTX) weekly which resulted in complete clinical and histological remission.

METHODS

Clinical data (Table 1). Two patients (E.C., I.H.) have previously been described elsewhere (Lange Wantzin *et al.*, 1983). Two patients (I.L., E.C.) had so-called classical benign lymphomatoid papulosis with recurrent papules. Patient I.L. in addition had parapsoriasis en plaque, of the large plaque type. Patient I.H. had a more 'malignant' type of lymphomatoid papulosis with recurrent large tumour-like lesions.

Routine laboratory investigations were normal in all three patients. In addition bone marrow, liver biopsy, and lymphography were normal in one patient examined (patient I.H.).

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TABLE 1. Clinical data on three patients with lymphomatoid papulosis

						MTX treatment		
Patient	Sex	Age (years)	Comments	Duration of disease (years)	Average no. of lesions (monthly)	Dosage (weekly)	Duration (months)	Average no. of lesions (monthly)
I.L.	F	67	Parapsoriasis en plaque	13	15	5 mg	5	0
E.C.	F	53	—	7	15	5 mg	18	1
I.H.	M	39	Splenectomy 13 years earlier	14	60	20–25 mg	15	0

Histology. There was a dense, deep and superficial dermal infiltrate consisting of small mature lymphocytes, eosinophils and neutrophils, and in addition large atypical lymphoid cells were found. Some mitoses and vasculitis of the small vessels with extravasation of erythrocytes were common findings. Patient I.H. also had a few Reed–Sternberg-like cells in the dermal infiltrate. Using monoclonal antibodies by the immunoperoxidase technique on skin sections, it was found that the majority of the cells were T-helper cells (Ralfkiaer, personal communication).

Previous treatment. Previously patients E.C. and I.H. had been treated with PUVA for 4 and 17 months, respectively. In both cases partial remission was obtained (Lange Wantzin & Thomsen, 1982) with shortening of the life cycle of the individual lesions and less papular lesions.

MTX treatment. MTX was given orally in weekly doses of 5 to 25 mg for 5–18 months (Table 1).

RESULTS

In all three cases MTX completely controlled the disease. The dosage needed, however, varied from 5 to 25 mg/week.

Patient I.L. was initially treated with 15 mg MTX weekly with prompt effect. After 2 weeks only one lesion could be observed and the patient has been maintained on 5 mg weekly without new lesions. The parapsoriasis en plaque lesions were unaltered.

Patient E.C. was initially given 10 mg MTX weekly, and after 1 month the development of new lesions had ceased. The patient was continued on 5 mg with only one small but histologically non-diagnostic lesion monthly.

Patient I.H. was initially treated with 15 mg MTX weekly without effect. The dosage was therefore increased to 25 mg weekly. On this dosage ten discrete folliculitis-like lesions have appeared monthly on the trunk and extremities, and on three occasions histological examination has shown only folliculitis without any features of lymphomatoid papulosis.

DISCUSSION

Two patients (I.L., E.C.) with 'classical' benign lymphomatoid papulosis and one patient (I.H.) probably with a primary cutaneous T-cell lymphoma have been treated with oral MTX weekly.

In two of the cases (I.L., I.H.) the lymphomatoid papulosis lesions disappeared completely and in one patient (E.C.) only one uncharacteristic papule occurred monthly.

Two of the patients (E.C., I.H.) have previously been treated with PUVA and achieved a partial remission (Lange Wantzin & Thomsen, 1982), while MTX had an immediate and convincing effect upon the skin lesions.

The risk of development of malignancy in some patients with lymphomatoid papulosis (Kawada *et al.*, 1969; Black & Wilson Jones, 1972; Dupont, 1973; Messenger, Marshall & Summerly, 1981; Scheen, Doyle & Winkelmann, 1981; Weinman & Ackerman, 1981; van der Putte *et al.*, 1982; Sanchez *et al.*, 1983) makes new diagnostic methods such as the use of monoclonal antibodies on skin sections and single cell DNA measurements important in identifying those cases that have a malignant potential (Lange Wantzin *et al.*, 1983; Ralfkiaer, personal communication). Although we do not know whether treatment of lymphomatoid papulosis modifies the natural history of the disease, effective treatment of early stages of potential malignant cases may be important in preventing malignant evolution. The present results of treatment with MTX in low dosage are promising in that a pronounced clinical and histological effect was observed. However, so far we have not been able to discontinue MTX treatment in these cases.

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Erythromelalgia following pergolide administration

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SUMMARY

Two patients are described in whom treatment of Parkinson's disease with the ergot derivative pergolide was associated with the development of erythromelalgia. The possible mechanism of pergolide-induced erythromelalgia is briefly discussed.

CASE REPORT

Case 1

A 67-year-old West Indian lady presented to the dermatology department with a 6-month history of an eruption on both shins. She was known to have had Parkinson's disease for 10 years, and for the past 2 years she had been on treatment with pergolide with good effect, having previously been treated with bromocriptine. She gave a past history of pulmonary tuberculosis and pulmonary and ocular sarcoidosis. Examination revealed a bilateral symmetrical erythema of both shins which were warm and slightly indurated. There were also numerous small mauve papules on the neck, shoulders and arms. Two skin biopsies taken from the shins showed marked focal capillary proliferation in the upper dermis and a minimal inflammatory infiltrate. Biopsy of one of the lesions on the arm revealed non-caseating granulomas consistent with sarcoidosis.

Case 2

A 68-year-old man presented to the dermatology department with an 18-month history of a hot erythematous eruption on the shins which had initially come and gone but which had latterly remained fixed. The appearance of the eruption was similar to that seen in case 1. This patient had been commenced on therapy with pergolide for his Parkinson's disease 1 year prior to the development of the eruption. He had previously been treated with L-Dopa, and was also taking allopurinol for gout.

Both patients had gained so much benefit from pergolide in terms of symptomatic relief of their Parkinson's disease that they declined to discontinue the drug.

DISCUSSION

Erythromelalgia (sometimes called erythermalgia) is an uncommon chronic disorder of unknown cause characterized by intermittent or persistent and sometimes painful erythema and heat of the extremities. Babb, Alarcon-Segovia and Fairbairn (1964) reviewed fifty-one cases seen at the Mayo Clinic over a 10-year period. They found that in some 40% of their cases there was an associated disorder, commonly a myeloproliferative one, the remainder being idiopathic. Michiels *et al.* (1980) reported that erythromelalgia was a common presenting feature of thrombocythaemia and that such cases, if untreated, could progress to peripheral gangrene.

The mechanism of erythromelalgia is unknown; Jorgensen and Sondergaard (1978) found excessive prostaglandin synthesis in lesional skin of two patients, but it is not clear whether this is the primary defect or a secondary phenomenon.

Erythromelalgia has not previously been reported in association with the use of pergolide, nor have any other cases been reported to the manufacturers (Lilly Research Centre Ltd.). It was however found to develop in seven of ninety-two patients with Parkinson's disease treated with bromocriptine (Calne *et al.*, 1978), an agent with structural and pharmacological similarities to pergolide. In these patients erythromelalgia was (as in the patients reported in this paper) found to develop only after many months of treatment.

Pergolide and bromocriptine are members of a class of drugs known as ergolines, being semisynthetic derivatives of the ergot alkaloids with dopamine agonist activity. They may be indicated for the treatment of Parkinson's disease, and, at lower doses, acromegaly and hyperprolactinaemia. Pergolide, by virtue of its long half-life, has been found to be of especial value in Parkinsonian patients suffering from diurnal oscillations in performance (Lieberman *et al.*, 1982).

Ergot is the product of the action of the fungus *Claviceps purpurea* on rye and other grains. The ergot alkaloids have powerful vasoconstrictor properties, and epidemics of burning and redness followed by gangrene of the extremities following ingestion of contaminated grain has been known since ancient times.

Bromocriptine may also produce peripheral vasoconstriction, Raynaud's phenomenon being found in some 40% of patients being treated with it for acromegaly (Wass *et al.*, 1977). The vasoconstrictor effect of ergot alkaloids is more marked on the post-capillary than the precapillary vessels (Mellander & Nordenfelt, 1970). This effect might account for the apparent paradox of the vascular dilatation seen in erythromelalgia being provoked by a vasoconstrictor such as an ergoline. It would be consistent with the beneficial effect of vasodilators in erythromelalgia, and the ineffectiveness of treatment with vasoconstrictors (Ratz, Bergfeld & Steck, 1979; Ozsoylu, Caner & Gokalp, 1979).

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Case Reports

Follicular ichthyosis

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SUMMARY

We describe four patients with congenital follicular hyperkeratosis, of whom three also had pseudoacanthosis nigricans and two had facial abnormalities. Skin specimens were studied by light microscopy, autoradiography, histochemistry and scanning electron microscopy, and the results were compared with those from patients with keratosis pilaris. The condition appears to constitute a distinctive form of ichthyosis in which the abnormal epidermal differentiation occurs mainly within the hair follicles.

The primary disorders of keratinization and desquamation which cause a persistent, generalized scaling of the skin surface are known collectively as the ichthyoses. Although in some patients with autosomal dominant ichthyosis there may be prominence of the follicular orifices, with slight projection of scale, it is unusual for this to be a major component of the clinical picture.

We present four patients in whom the predominant clinical manifestation was the presence of horny follicular projections, present since early childhood. Associated abnormalities accompanying the characteristic skin changes distinguish this disorder from other conditions characterized by follicular hyperkeratosis, and we suggest that it represents an uncommon, distinctive subgroup of ichthyosis.

One of these patients has been reported previously (Waddington & Marks, 1978).

METHODS

Since 1976, four patients with generalized follicular hyperkeratosis have been examined and investigated in our clinic. Histological sections from these patients were reviewed and compared with those obtained from three patients with keratosis pilaris. In addition to examining routine paraffin-embedded and haematoxylin and eosin stained histological sections, small fragments of skin were obtained using a 4 mm punch biopsy. Pieces were then incubated in Eagle's minimal essential medium in the presence of tritiated thymidine (2 μCi/ml) for 4 hours, after which the skin was prepared by standard autoradiographic techniques.

Enzyme histochemical techniques were employed to demonstrate the non-specific esterase (NSE) reaction (Holt, 1952), the glucose-6-phosphate dehydrogenase (G6PDH) reaction, and the succinic dehydrogenase (SDH) reaction (Chayen, Bitensky & Butcher, 1973).

Surface replicas of representative sites in both groups were obtained, using silicone rubber impression material (Silflo) and DPX mounting medium to obtain 'positives', and examined under a scanning electron microscope (SEM).

RESULTS

The clinical details of the four patients are shown in Table 1. All patients had generalized follicular hyperkeratosis (Fig. 1), often more prominent on the head and neck. Three patients had acanthosis nigricans-like changes in the axillae with hyperpigmentation and skin thickening, and two had comedo-like lesions on the dorsal aspects of the fingers (Fig. 2) and cheeks. Two patients had abnormalities of dental occulsion, producing a similarity in the overall shape of the face (Fig. 3). The one female patient also had white hyperkeratotic patches on the buccal mucosa.

One patient had a strong family history of the condition (Fig. 4), the more severely affected members having reduced visual acuity, with affected males suffering premature hair loss. One other patient gave a personal history of asthma and allergic rhinitis.

Histological examination of sections stained by standard techniques with haematoxylin and eosin showed marked follicular hyperkeratosis (Fig. 5) with normal interfollicular epidermis. In particular there was a normal granular layer in the inter-follicular areas. The hyperkeratosis within the follicles consisted of compact, orthokeratotic stratum corneum, with an increase in the granular cell layer, and appeared to extend deep within the follicle (Fig. 6). In contrast, in

TABLE 1. Follicular ichthyosis—clinical details

	Patient 1 (S.E.)	Patient 2 (J.M)	Patient 3 (T.T.)	Patient 4 (A.P.)
Sex	Male	Male	Female	Female
Date of birth	18.3.54	7.2.64	26.9.55	16.12.67
Age of onset	18 months	3 years	Birth	Birth
History of atopy	No	No	No	Asthma Allergic rhinitis
Family history	Keratosis pilaris in father	Strong	No	No
Worst involved sites	Cheeks Ears Neck Upper trunk Arms	Limbs Cheeks	Uniform	Cheeks Ears Neck Forehead Chin
Comedo-like lesions	Cheeks Dorsum fingers	None	None	Cheeks Temples
Acanthosis nigricans-like changes	Axillae	None	Axillae	Axillae
Dental malocclusion	No	No	Yes	Yes

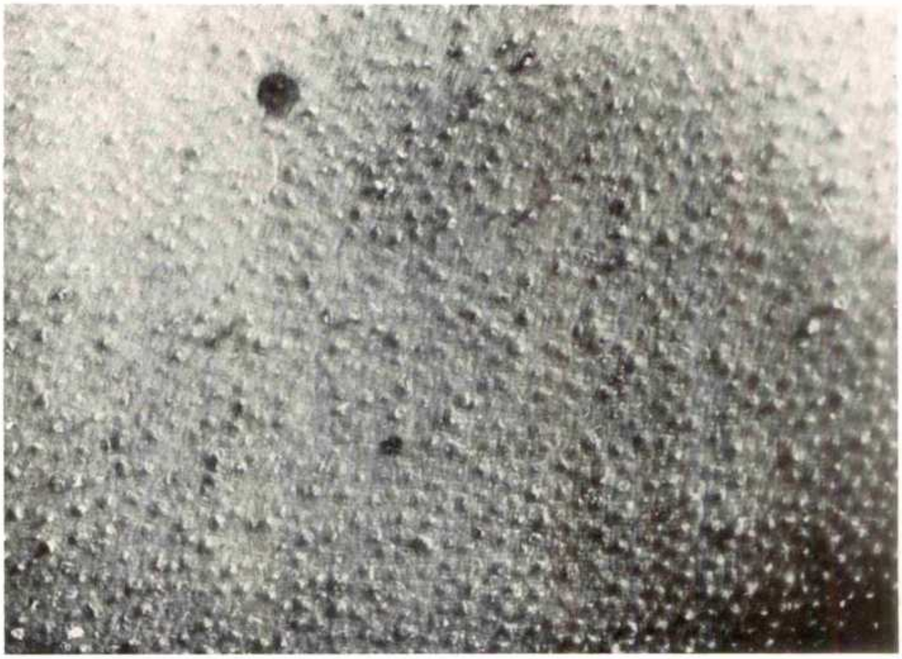

FIGURE 1. Follicular hyperkeratosis.

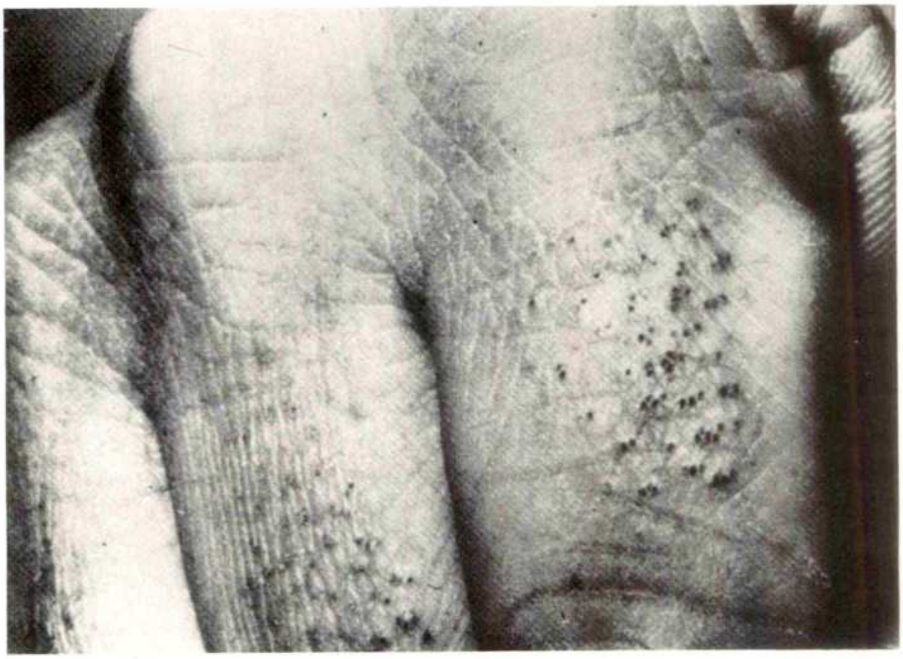

FIGURE 2. Comedo-like lesions on fingers.

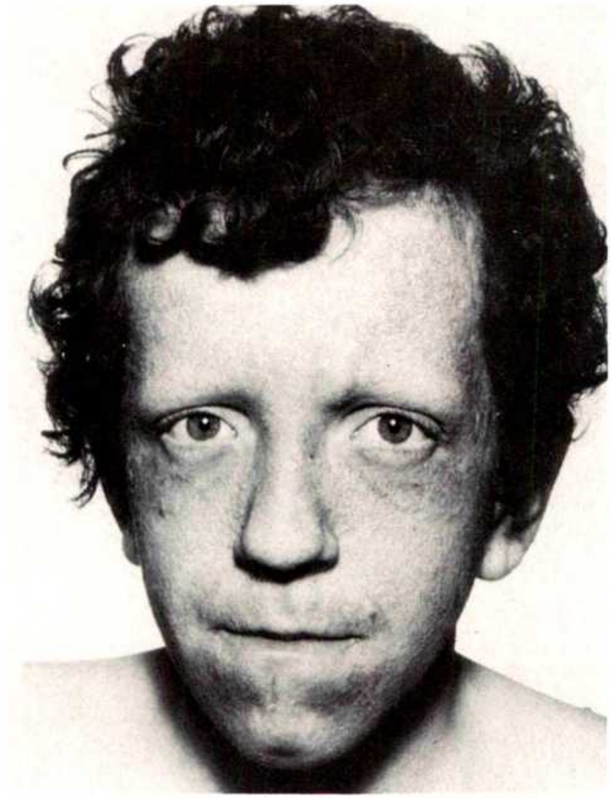

FIGURE 3. Facial appearance of patient 3.

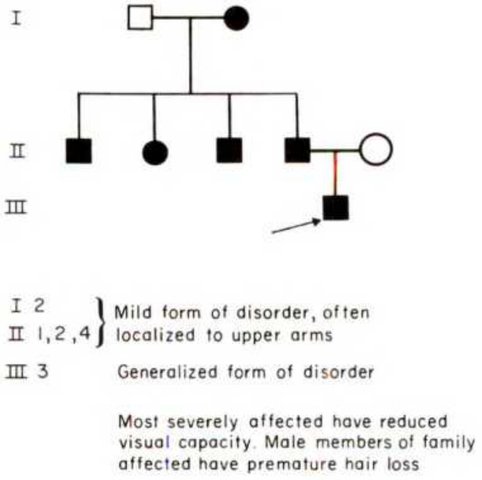
I
II
III
I 2
II 1,2,4
Mild form of disorder, often localized to upper arms
III 3
Generalized form of disorder
Most severely affected have reduced visual capacity. Male members of family affected have premature hair loss

FIGURE 4. Inheritance of follicular ichthyosis in patient 2 (J.M.).

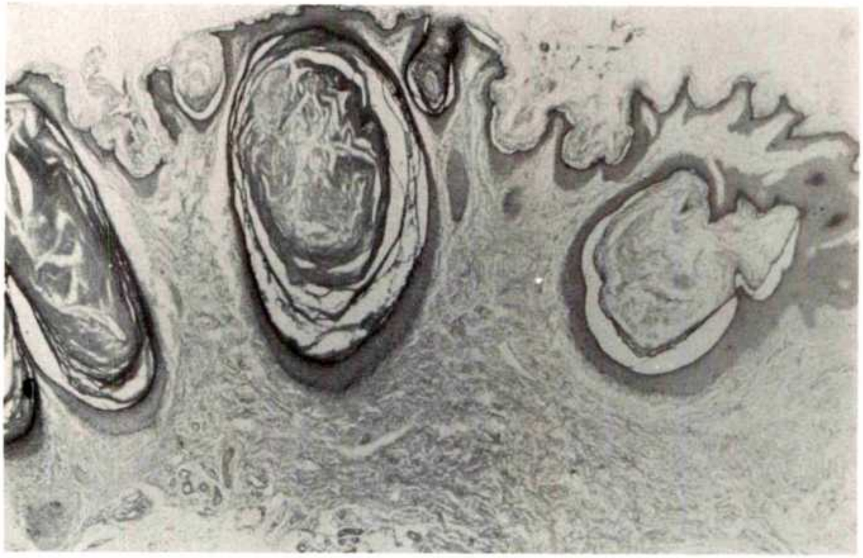

FIGURE 5. Photomicrograph of follicular ichthyosis showing marked follicular hyperkeratosis with relatively normal epidermis between follicles.

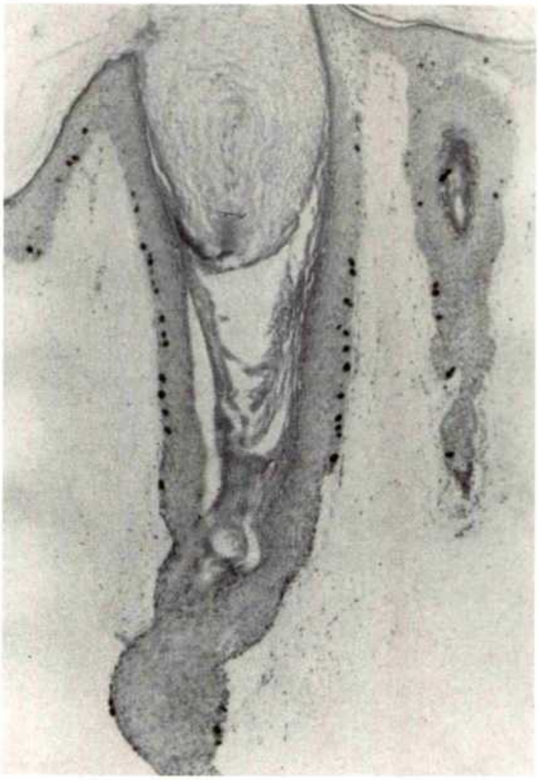

FIGURE 6. Autoradiograph of follicle in follicular ichthyosis, demonstrating both normal labelling and hyperkeratosis extending deeply within the follicle.

TABLE 2. Tritiated thymidine autoradiographic labelling indices

Patient	Diagnosis	Labelling index*
J.M.	Follicular ichthyosis	6·0
T.T.	Follicular ichthyosis	7·2
A.P.	Follicular ichthyosis	5·9
L.S.	Keratosis pilaris	5·2
A.B.	Keratosis pilaris	6·3
M.N.	Keratosis pilaris	8·5

* Percentage of labelled basal and suprabasal cells as a proportion of basal cells.

keratosis pilaris the follicular hyperkeratosis was confined to the infundibular region, and consisted of a more open, 'basket-weave' type of stratum corneum, the granular cell layer being normal. In both follicular ichthyosis and keratosis pilaris no inflammatory cell infiltrate was seen in the dermis.

Autoradiography showed the sites of epidermal cell nuclear labelling to be normal in both groups of patients. Labelling indices were within the normal range for our laboratory (4–8%) and there was no clear difference between the two groups (Table 2). The hair follicles in both

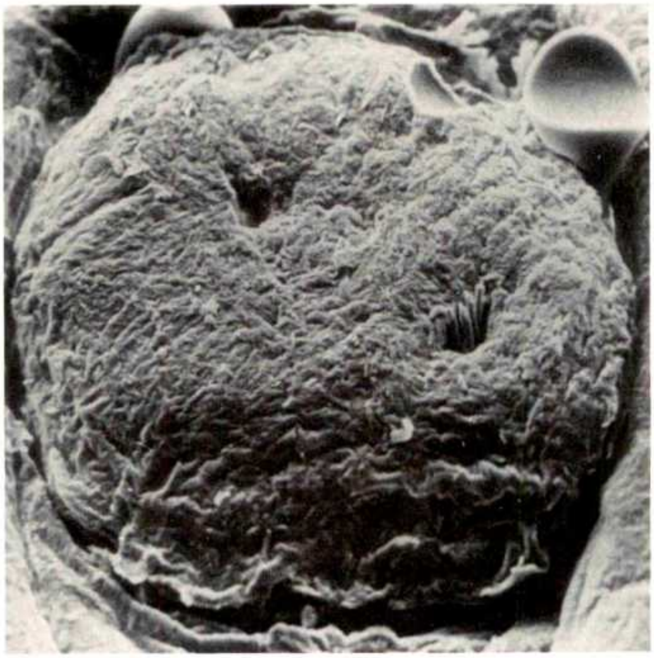

FIGURE 7. Scanning electron photomicrograph in follicular ichthyosis, showing compact horn plugging a hair follicle, with abnormal hairs protruding through the horn.

groups demonstrated the expected labelling of the external root sheath, with labelling indices of approximately 20%, again within our normal range, (Fig. 6).

Scanning electron microscopy of surface replicas did demonstrate a difference between the patients with follicular ichthyosis and those with keratosis pilaris. In the former group a compact horn was to be seen plugging the follicles, displacing abnormal hairs to one side of the follicle (Fig. 7). Occasionally abnormal hairs could be seen protruding through the compact horn. In the patients with keratosis pilaris, there was only a small amount of horny material within the follicles, surrounding normal hair (Fig. 8).

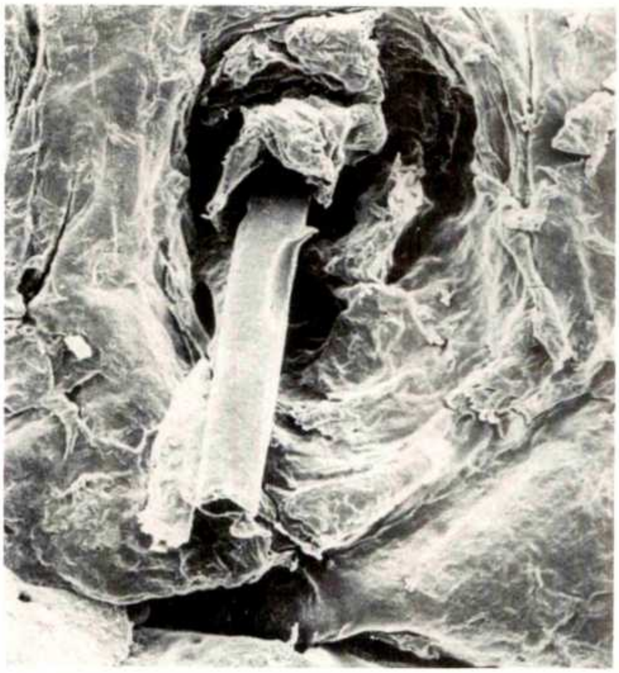

FIGURE 8. Scanning electron photomicrograph in keratosis pilaris, showing sparse, loose horny material within a hair follicle which contains a normal hair.

Enzyme histochemical techniques to demonstrate the changes taking place during epidermal differentiation showed no apparent difference between the patients with follicular ichthyosis and those with keratosis pilaris, neither group deviating from normal. However, in one of the two patients treated with etretinate (Tigason®), enzyme histochemical tests were performed both before and during treatment with the drug, and there appeared to be a decrease in formazan reaction product in the glucose-6-phosphate dehydrogenase reaction during treatment. This is similar to changes seen in other ichthyotic conditions treated with etretinate (Pearse *et al.*, 1983). Both patients treated with etretinate improved considerably whilst taking the drug but relapsed rapidly when it was withdrawn. The female patient has been so impressed with the effect of etretinate upon her skin that she prefers to tolerate a generalized pruritic, urticarial eruption due to the drug, rather than stop taking it.

DISCUSSION

The term follicular ichthyosis has been used previously in clinical descriptions. Robert Willan, in his treatise 'On Cutaneous Diseases' comments that in some areas the scales may be 'small, rounded, prominent or papillary in patients with ichthyosis'. White, in his description of what has become known in Europe as Darier's disease, used the term (White, 1889), as Domonkos *et al.* (1982) also did in their discussion of keratosis pilaris, although Brocq (1890) did not use it. One of the four patients discussed in this paper has been previously reported as a 'Follicular ichthyosiform disorder' (Waddington & Marks, 1978).

Writing about keratosis pilaris, Brocq comments that although imperfectly described, it was well known to what he terms the 'classical authors'. In his paper he discusses both the common form, and the less common *keratose pilaire rouge*, and in addition to its occurrence on the posterior aspect of the arms and the outer aspect of the thighs, comments that uncommonly the lesions may be seen on the trunk, or even upon the face. On the face the appearance is said to be most usually that of *keratose pilaire rouge*. A passing reference to a generalized form of keratosis pilaris is made by Ebling and Rook (1979), with an illustration. A number of variants of keratosis pilaris have been described, usually in association with atrophy and scarring (Rook & Ebling, 1979) and although case IV showed poor eyebrow formation (Fig. 3), there was no associated erythema, atrophy or scarring.

Keratosis pilaris is said to be associated with a number of conditions, including deficiencies of vitamin B_2 and vitamin C, hypothyroidism, and Cushing's syndrome or ACTH administration (Forman, 1954). Hill and Sulzberger (1935) claim a association with atopic eczema, but this has been challenged by Ofuji and Vehara (1973). However, such reports of 'associated' conditions are not unexpected in a condition said to affect 80% of 14-year-old girls and 20% of adult women. With the exception of atopy in one patient, no such associations were found in our patients with follicular ichthyosis.

A number of other conditions may demonstrate a marked follicular component clinically (Table 3) or even a 'pseudo-follicular' appearance, as in lichen nitidus. However, these conditions are readily distinguishable from follicular ichthyosis both clinically and histologically.

We believe that the patients described have a distinctive disorder of keratinization in which the brunt of the abnormality occurs in the hair follicles. The association with the acanthosis

TABLE 3. Dermatoses with a follicular distribution

Keratosis pilaris and variants
Folliculitis decalvans
Lichen spinulosus
Lichen planopilaris
Pityriasis rubra pilaris
Darier's disease
Phrynoderma (vitamin A deficiency)
Vitamin B_2 deficiency
Vitamin C deficiency
Kyrle's disease
Follicular psoriasis

nigricans-like changes, and the characteristic facies suggests that these patients form a distinct subgroup of ichthyosis, with genetic linkage between the various clinical manifestations.

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Multiple Beau's lines due to dysmenorrhoea?

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SUMMARY

Four young women who complained of nail ridging, were found to have several evenly spaced Beau's lines. The lines appear to be related to their menstrual cycles and each one had severe dysmenorrhoea. Linear nail growth was not impaired.

Beau's initial description of transverse ridging of the nails related the phenomenon to acute febrile illnesses (Beau, 1846). Since then the lines have been reported in a number of different conditions (Baran & Dawber, 1984). It has been suggested that during the phase of formation of these transverse depressions, linear nail growth is arrested, but there are no records of nail growth measurement during these episodes. We report here four cases in which multiple, regular Beau's lines occurred in relation to severe dysmenorrhoea.

METHODS

Four women, aged 16, 16, 18 and 19 years, respectively, were seen in the dermatology out-patient clinic. They had all been aware of transverse nail lines being present for more than 1 year. Systemic enquiry revealed no evidence of significant disease other than severe dysmenorrhoea with each period for at least 1 year. All the women had ten fingernails affected; one had the two great toe nails and one had all her toe nails involved. The Beau's lines were evenly spaced out (Fig. 1) and no one had more than four per nail. None of the women showed evidence of nail biting or excessive cuticle damage through manicuring or other trauma. No patients had evidence of skin disease or general disorder; haemoglobin, white cell count, urea, electrolytes and liver function tests were all normal.

Linear nail growth was measured using the method described by Dawber (1970); the mean growth rate over a 4-week period was calculated using the index finger in each case. In two patients it was possible to take a further measurement of growth rate over a 4-day period of dysmenorrhoea (this is the shortest period over which measurement is accurate).

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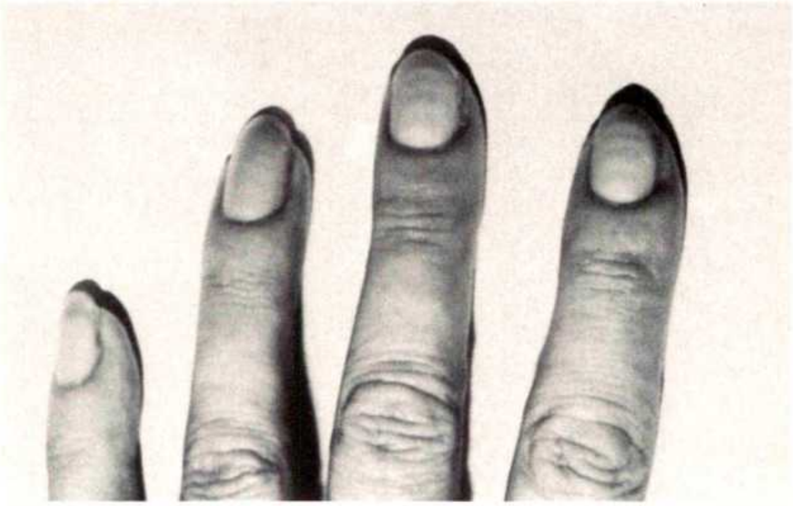

FIGURE 1. Beau's lines in a woman with severe dysmenorrhoea.

RESULTS

The mean daily growth expressed in millimetres was: patient 1, 0·104; patient 2, 0·096; patient 3, 0·144; patient 4, 0·115. During 4 days of dysmenorrhoea, the rates were: patient 1, 0·101 mm and patient 2, 0·099 mm.

These rates of linear growth fit with the conclusion that the Beau's lines occurred monthly.

DISCUSSION

Beau's lines may affect only the thumb and big toe nails, or all twenty nails. Recurrent local disease, e.g. eczema or systemic illness, may produce repeated transverse lines.

Our studies have shown no evidence of impaired linear nail growth. It is interesting to postulate that the mechanism producing Beau's lines also underlies the reduction of hair shaft diameter found during illness (Matsuura, 1902; Pinkus, 1928; Sims, 1967). Constriction of the hair shaft has been reported after the administration of cytostatic agents. Cyclophosphamide, methotrexate and actinozine D can all have this effect (Crounse & Van Scott, 1960). Such disease or treatment is believed to inhibit hair bulb mitotic activity without causing anagen effluvium. It does not result in decreased linear growth but causes a focal narrowing of the hair-shaft.

In view of the normal linear nail growth in our patients, it seems likely that Beau's lines, like the hair constriction of Pinkus (1928) and Sims (1967) are due to inhibition of nail matrix mitotic rate and thinning of the nail plate. The most severe inhibiting influences on hair cause very marked narrowing and fracture. By analogy, the severest influence on nail growth is latent onychomadesis.

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Comment

AZELAIC ACID

Azelaic acid (1,7-heptanedicarboxylic acid, $HO_2 \cdot C(CH_2)_7 \cdot CO_2H$) is a naturally occurring straight-chained 9-carbon atom (C_9) saturated dicarboxylic acid with a molecular weight of 188·22, and a melting point of 105·5°C. It can be prepared by disruptive oxidation of ricinoleic acid, and occurs in rancid oleic acid. It is non-toxic and non-teratogenic (Mingrone *et al.*, 1983), and when administered orally in man (up to 20 g a day is tolerated), about 60% is excreted as such in the urine within 12 hours; it is partially metabolized by β-oxidation (Passi *et al.*, 1983). The 15% sodium salt has been administered intravenously, intra-arterially, and intralymphatically by continuous infusion for up to a week without deleterious local or systemic effects (Nazzaro-Porro *et al.*, 1983b).

Interest in the biological activity of azelaic acid arose originally out of studies on skin surface lipids and the pathogenesis of hypochromia in *Pityriasis versicolor* infection (Caprilli *et al.*, 1971; Caprilli, Nazzaro-Porro & Passi, 1973). It had been generally thought that the hypopigmentation in this condition was due to filtering out of UV irradiation by the fungus in the stratum corneum, but ultrastructural examination of biopsies revealed damage to melanocytes ranging from swelling and vacuolation of mitochondria to extensive degeneration of some cells (Breathnach, Nazzaro-Porro & Martin, 1975). The lipophilic and lipido-dependent nature of *Pityrosporum* and the highly modified pattern of skin surface lipids associated with its presence (Caprilli *et al.*, 1971, 1973; Boniforti *et al.*, 1973; Passi *et al.*, 1977) suggested that some metabolic products of lipid nature might be the cause of this damage. This hypothesis seemed to be confirmed when it was shown that Pityrosporum is capable of oxidizing unsaturated fatty acids, giving rise to lipid substances identified as dicarboxylic acids, and that those diacids with chain lengths from C_8 to C_{13} were competitive inhibitors of tyrosinase *in vitro* (Nazzaro-Porro & Passi, 1977, 1978a; Nazzaro-Porro *et al.*, 1979b). It seemed reasonable to assume that the hypochromia in *Pityriasis versicolor* might be due to the anti-tyrosinase activity of these diacids, and the possibility arose that they might be beneficially applied to the treatment of hyperpigmentary disorders.

A cream containing 15–20% azelaic acid (C_9) was prepared and, when applied topically, proved effective in the treatment of melasma and post-inflammatory melanosis, leaving no residual hypochromia (Nazzaro-Porro & Passi, 1978b). Azelaic acid was originally chosen in preference to other diacids (such as, for instance, dodecanedioic acid, C_{12}, which has similar effects) because of its low cost, and the relative ease with which it can be incorporated into creams for topical application. A positive beneficial effect was also observed following application of the cream to cases of lentigo maligna (Nazzaro-Porro *et al.*, 1979a, 1982). To date, fifty-one cases of lentigo maligna have been treated by us during the past 7 years. In all cases, clinical and histological resolution of lesions was achieved, and this was confirmed in some cases by ultrastructural examination of serial biopsies taken during the course of treatment. These showed destruction and disappearance of abnormal melanocytes, regeneration of a normal dermis and epidermis, and repopulation with normal melanocytes. Some relapses occurred, but these responded fully to re-application of the cream. No residual hypochromia was observed,

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nor was there any scarring. This beneficial effect of topical azelaic acid on lentigo maligna has recently been confirmed by Leibl *et al.* (1983) in four cases, and in our opinion, it provides a most satisfactory form of therapy, especially in early cases and for extensive long-standing lesions in older patients. We are aware of a body of opinion that maintains surgery to be the only justifiable treatment for lentigo maligna. However, surgical treatment may be constitutionally inapplicable, and its results can often be cosmetically unacceptable. Those who would advocate it as the only treatment must take into account the fact that lentigo maligna is a relapsing condition, and that surgery, by its very nature, can be extemely difficult to apply a second time.

Ertle, Wiskemann & Jänner (1983) treated malignant lentigines in a patient with xeroderma pigmentosum with azelaic acid for 3 months, and, while somewhat disappointed with the clinical results, they reported 'impressive alterations' on histological and electron microscopic examination. These included re-establishment of 'a well-defined dermal-epidermal junction, diminution of pigmentation, as well as disappearance of melanoma cells from the epidermis'. These observations closely parallel ours in lentigo maligna (Nazzaro-Porro *et al.*, 1979a).

Application of azelaic acid cream has no effect on normal skin of whatever degree of pigmentation, nor has it any effect on freckles, senile lentigines, or pigmented naevi. Pathak *et al.* (1979) reported no effect on the skin of normal pigmented guinea-pigs. Clinically, therefore, we have concluded that azelaic acid exerts its effect only, or predominantly, upon hyperactive or abnormal melanocytes. This is borne out by the demonstration that melanocytes in mixed cultures of epidermal cells were not damaged by addition of 150–200 μg/ml of azelaic acid (about 10^{-3} M) to the medium (Breathnach *et al.*, 1979).

Studies on experimental animals inoculated with Harding–Passey melanoma revealed that oral, subcutaneous, or intra-peritoneal administration of azelaic acid resulted in significant retardation of tumour growth and prolongation of life, and ultrastructural examination revealed degeneration and disappearance of malignant melanocytes (Nazzaro-Porro *et al.*, 1979b). These results led us to investigate the effect of the diacid on human primary cutaneous malignant melanoma, after having established that it is neither toxic nor teratogenic (Mingrone *et al.*, 1983).

Observations were made on twenty-nine patients awaiting surgery for primary superficial spreading and nodular melanoma without lymph node involvement (Nazzaro-Porro *et al.*, 1980, 1983c, 1983d, 1983e). They were given 10–15 g azelaic acid orally each day, in addition to topical treatment with a 20% cream for periods of 2 weeks to 8 months. All but two patients showed positive results, manifested by progressive loss of pigment, regression of advancing edge of the lesion, and flattening of nodular areas. Histological and ultrastructural examination of lesions following excision revealed a cytotoxic effect on the abnormal melanocytes, and regeneration of a normal epidermis and dermis. In eight patients, treatment for 6 to 8 months led to complete regression of the tumour, clinically, histologically, and ultrastructurally. These results clearly show that azelaic acid has a biological and cytotoxic effect on the human malignant melanocyte. They cannot, however, be presented as a claim that azelaic acid *per se* is an effective treatment for primary cutaneous melanoma. They do, however, justify further studies, both clinical and laboratory, to determine what its ultimate role, possibly in combination with other treatments, may be.

The positive effect on primary melanoma of topical application has led to enquiries concerning systemic application of azelaic acid in cases of disseminated metastatic melanoma. Intravenous infusion of a 15% solution of the sodium salt has been reluctantly tried at the behest of physicians as a last resort treatment in a number of terminal cases, with, as expected, little observable result. The problem with systemic treatment lies with delivering and maintaining a sufficiently high concentration of the diacid at the sites of lesions for a sufficiently long period. Only a very low concentration of azelaic acid may reach internal lesions after oral or intravenous administration. As shown by studies on the metabolism of the diacid (Passi *et al.*, 1983), in man the serum levels of azelaic acid peak at 2–3 hours after oral administration of 5 g, reaching only 74 μg/ml, i.e. a concentration of about 4×10^{-4} M. About 60% of the ingested diacid is eliminated as such in the urine, and the remaining part is β-oxidized. Until the molecule can be modified in order to obtain azelaic acid derivatives active at lower concentration, intravenous administration holds out little promise of success. It might, however, be worthwhile trying for localized metastatic disease, as for example, in the form of isolated whole limb closed-circuit perfusion. The same considerations apply even more so to oral medication, and it is not surprising that it has been reported as ineffective at 12 g per day for a maximum of 12 weeks in the management of ocular and adnexal melanoma (Willshaw & Rubinstein, 1983). We now believe that in our originally reported cases (Nazzaro-Porro *et al.*, 1980), any effect of oral administration was probably minimal. In this general context, we can refer to a patient who presented with axillary nodes exhibiting histologically confirmed malignant melanoma without any discoverable primary lesion. He was given a 15% solution of the sodium salt of azelaic acid by continuous intralymphatic infusion for 4 days, after which there was a complete surgical clearance of axillary glands, which were then reported to exhibit no melanoma histologically, though some was seen on electron microscopy. He remains well with no recurrence 3½ years after. No more than has been said above should be read into this case, but it does illustrate the fact that intralymphatic infusion is a practicable approach for possible treatment of involved lymph nodes by azelaic acid, or a modified and more active form of it which may become available in the future.

The idea that dicarboxylic acids operate primarily by anti-tyrosinase activity, and that they are specifically cytotoxic for melanocytes, came under question with the demonstration that a 15% cream applied for 3–9 months is beneficial in cases of acne vulgaris, including severe nodulo-cystic forms (Nazzaro-Porro *et al.*, 1983b). Metabolic studies (Passi *et al.*, 1984b), showed that dicarboxylic acids are partially metabolized by β-oxidation. This means that they enter into mitochondria, and are transformed by β-oxidative enzymes into acetyl-CoA and malonyl-CoA. The fact that they enter mitochondria led to the suggestion that they could interact with enzymes other than those responsible for β-oxidation, e.g. some oxido-reductases of the respiratory chain. It emerged from studies on isolated rat liver mitochondria (Picardo *et al.*, 1983; Passi *et al.*, 1984a) that at concentrations of 10^{-3} M to 10^{-2} M, dicarboxylic acids from C_8 to C_{13} are capable of inhibiting cell respiration, and that they are competitive inhibitors of mitochondrial oxido-reductases such as NADH dehydrogenase, succinic dehydrogenase, and reduced ubiquinone-cyt-c-oxido-reductase. There is therefore a parallel between their reactions with mitochondrial oxido-reductases and with tyrosinase, which is also an oxido-reductase.

Analytical ultrastructural autoradiographical studies on normal melanocytes (Breathnach *et al.*, 1983) and murine melanoma cells (Ward *et al.*, 1984), incubated in tissue culture with radioactive dicarboxylic acid (C_{12}), tend to confirm the enzymatic studies. Significant radioactivity was found in mitochondria and nuclei, but not in organelles associated with tyrosinase location or melanogenesis. Parallel biochemical studies confirmed that the diacid underwent β-oxidation, which occurs only in mitochondria. Other experiments of ours (unpublished, but reported at the XIIth International Pigment Cell Conference Giessen, September, 1983) have shown that azelaic acid (at concentrations of 10^{-2} M and greater) significantly reduces proliferation of Harding–Passey and Cloudman melanoma cells over 3 days in culture. The mitochondria of the cells exhibited massive swelling and destruction of cristae, but no damage to other cytoplasmic organelles or to the cell membrane was seen. These overall results clearly suggest that the cytotoxicity of dicarboxylic acids is not related to anti-tyrosinase activity *per se*, but is primarily due to inhibition of mitochondrial respiration, and very probably also to inhibition of DNA synthesis in both mitochondria and nucleus (Breathnach *et al.*, 1983; Ward *et al.*, 1984). This fits in well with Wilkie's (1979) proposition that some anti-cancer drugs act primarily against mitochondria, and suggests that dicarboxylic acids may be equally effective against hyperactive or malignant cells other than melanocytes. Preliminary experiments with normal and abnormal lymphocytes in tissue culture suggest this may well be so, and the effect of azelaic acid on other tumour cells in tissue culture is at present being investigated. Clinically, a positive effect on solar keratosis has been observed. The probability that azelaic acid affects DNA synthesis makes it worthwhile to investigate whether it has an anti-viral activity. In fact, colleagues of the Department of Virology at St Mary's Hospital report that it has definite anti-viral activity *in vitro*, and there is a possibility that it might abort recurrent episodes of labial herpes.

The mechanism of action of azelaic acid on acne is currently under investigation. Because of its anti-oxidase activity, azelaic acid might be thought to interfere with sebaceous secretion, follicular epithelium hyperplasia, or microbial activity. Preliminary studies (unpublished) carried out on the sebaceous flank organ of the Golden Hamster, plus an NADPH-generating system and testosterone, showed an inhibition of the activity of 5-alpha reductase, probably due to a competitive inhibition of the oxidation of its NADPH CO coenzyme. We also have evidence that azelaic acid inhibits the growth of aerobic bacteria and the anaerobic *Propionibacterium acnes* (Nazzaro-Porro *et al.*, 1983b). In addition, thin-layer chromatography performed on skin surface lipids from skin areas treated with azelaic acid cream showed a marked reduction (about 60%) of the free fatty acid fraction as compared with the control areas, indicating an inhibition of bacterial lipase activity. Marsden and Shuster (1983), in a brief account of a study of nine patients over a period of 6 weeks, reported that azelaic acid cream had no sebostatic effect. As regards this question of measuring sebum excretion rate, however, it must be remembered that azelaic acid itself is a fatty diacid and, depending upon the technique used, could affect the measurement.

It has been emphasized that clinically, azelaic acid has no effect on normal melanocytes. Yet in *Pityriasis versicolor* where, presumably, normal melanocytes are involved, depigmentation and damage to melanocytes is evident. This is a matter that has always required explanation, and one can now be put forward. It has been shown (Nazzaro-Porro *et al.*, 1983a) that *Pityrosporum* cultured with a sebum-like mixture has a high lipoxygenase activity, and that lipoperoxidation involves not only unsaturated fatty acids, which lead to production of dicarboxylic acids, but also squalene and cholesterol. During the process, lipid radicals (lipoperoxy-radicals, lipohydroperoxides, etc.) are produced, all of which are known to be highly unstable, reactive, and cytotoxic. It is probably these substances, and not the more stable dicarboxylic acids which are responsible for the hypochromia and damage to melanocytes in *Pityriasis versicolor*.

This still leaves the question as to why dicarboxylic acids damage hyperactive and malignant melanocytes, but not normal cells. We have evidence (unpublished) that one factor, at any rate, concerns permeability of the cell membrane to the diacids. Normal cells exhibit a limited permeability and diacids penetrating the cell membrane undergo complete metabolism by β-oxidation. With neoplastic cells, penetrability is much higher, about three times, with resulting higher intracellular concentration. All the dicarboxylic acids probably cannot be dealt with by β-oxidation, and the excess is available for inhibiting mitochondrial oxido-reductases.

It is clear that azelaic acid, and other dicarboxylic acids, have significant biological activities, and definite potential as therapeutic agents for a number of conditions. Clinical investigations are being carried out at a number of centres in continental Europe and, in general, our results with lentigo maligna and acne are being confirmed. A randomized double-blind clinical trial of its effect on acne compared with other treatments is being organized.

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Correspondence

The effect of large doses of H1 antagonists in urticaria

SIR, In the past the dose of histamine H1 antagonists given to a patient has been largely limited by the development of side-effects. Now there are H1 antagonists virtually without sedative side-effects it is possible to give very large doses indeed.

Patients attending the Urticaria Clinic were treated where appropriate with the usual dose of terfenadine, namely 120 mg daily. In twenty-four patients the response was not sufficient and weals were still present. These patients had ordinary chronic urticaria, some with associated angio-oedema, and two also had pressure urticaria. There were nine males and fifteen females, mean age 46 (range 15 to 64) years.

The dose of terfenadine was steadily increased at 2- to 4-week intervals to a daily dose of 240 mg (in six patients), 360 mg (in six patients), 480 mg (four patients), 600 mg (two patients), 720 mg (three patients), 840 mg (one patient), and 960 mg (two patients).

In most of the patients, increasing the daily dose to 240 mg slightly increased the effects of the antihistamine, but beyond this dose there was little or no improvement. Even with doses of 360 mg and more, weals continued to develop. It would seem, therefore, that mediators which cannot be antagonized by a H1 blocker such as terfenadine play an important role in the urticaria weal formation.

None of the twenty-four patients had any drowsiness. One patient on 960 mg daily complained of slight dizziness, but some complaint might well be expected from a patient required to swallow sixteen tablets per day, and she also complained of similar effects on an average dose of an older antihistamine.

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The association between lichen sclerosus et atrophicus and auto-immune diseases in males is not proven

SIR, In their paper 'Lichen sclerosus et atrophicus and autoimmune disorders' (*British Journal of Dermatology* 1983, **109**, 661–664) Meyrick-Thomas, Ridley and Black claim to have demonstrated a highly significant difference ($P<0{\cdot}001$) in the incidence of autoimmune disorders in twenty-five male patients with lichen sclerosus et atrophicus compared with twenty-five age-matched control patients who were attending the Dermatology Department for other reasons. This claim cannot be substantiated by the figures presented. Data of this type should be analysed by a contingency table, in which case the most powerful test would be Fisher's exact test because of the low expected frequency of less than five in two of the cells (Siegel, 1956). By this analysis the difference between the two groups just fails to reach significance in a two-tailed test ($P=5{\cdot}02\%$) when alopecia areata and vitiligo are combined (five of twenty-five patients against zero of twenty-five controls). The data completely fails to reach significance for alopecia areata alone (three of twenty-five against zero of twenty-five controls) where $P=25\%$, and for vitiligo alone (two of twenty-five against zero of twenty-five controls) where $P=49\%$.

The weakness of this study lies in the small number of patients and controls observed which has made a statistical type I error highly likely (the inability to demonstrate a true difference when one exists). Unfortunately, the authors go on to compound this felony by comparing their own incidence rates with figures for the 'normal population' by quoting data derived from very diverse sources:

(a) The figures for alopecia areata were obtained from a mixture of dermatological and orthopaedic patients in Sheffield up to four decades previously.

(b) The population statistics for vitiligo were obtained from a general population survey conducted on Bornholm Island in Denmark.

(c) The figures quoted for the incidence of autoantibodies in the general population were derived from the male population of Australia.

This paper may be said to have reached the 'finishing line' in the great 'Senior Registrar paper-chase', but it is by no means a credit to the designers of the study who, by a little applied statistical thought, could have achieved much more. Despite the attempts of many [including the Editor of this Journal (Burton, 1975)] to promote statistical awareness it is distressing to see that magical '*P*' values, however derived, seem to bamboozle authors and readers into believing that which common sense dictates to be plainly untrue.

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Reply

SIR, We thank Dr Simpson for his letter and are grateful to him for pointing out the error in the statistical analysis of our results. A statistician advised that the data from our two groups should be compared by the test of proportions, and from his application of that test our results were interpreted as being of statistical significance. In the light of Dr Simpson's comments we now appreciate that Fisher's exact test is the correct statistical technique for analysis of the data and agree that by application of this test to our results, $P = 0.0502$ (which very narrowly misses statistical significance). In analysing results we acknowledge the importance of the choice of statistical test and its correct application; however, it may not necessarily be as simple as Dr Simpson's 'common sense' suggests for those of us who are less statistically knowledgeable always to appreciate when an inappropriate statistical significance has been obtained as a result of the incorrect analysis of a given set of data.

With regard to Dr Simpson's observation that, when taken alone, the data for alopecia aretata and vitiligo are not statistically significantly different between our patient and control groups, we did not suggest that they were.

The only published data based on large population surveys of the incidences of alopecia areata, vitiligo and autoantibodies in Caucasian populations, were provided by the references cited; these references were used to provide both data regarding the sex ratios of alopecia areata and vitiligo, and independent figures against which to put our results into perspective and were not used for any form of statistical comparison.

As so often occurs when making clinical observations on a relatively uncommon disease, a larger group of patients to study would be most welcome; we hope that we will be able to enlarge our study, and thereby strengthen our case, for believing there to be an association between lichen sclerosus et atrophicus and autoimmune diseases in men.

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Prevention of dithranol bath stains

SIR, Drs Freeman and Warin report that dithranol stains plastic and enamel bath surfaces (*British Journal of Dermatology* 1984, **110**, 246). By the time the patients complain about bath stains, it is too late, and nothing will remove them. However, if the patient is shown before treatment how to clean the bath after each application, then staining can be prevented.

We demonstrate the use of bleach before the beginning of treatment to each patient attending the department. However, commercially available cleaners such as Jif® (Lever Brothers) and Flash® (Procter & Gamble) are also effective and the manufacturers claim that they are safe to use on plastic surfaces. We also instruct the patients to remove surplus ointment with a tissue before bathing.

I hope these points will be of practical assistance.

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Remission rates in acne patients treated with various doses of 13-*cis*-retinoic acid (isotretinoin)

SIR, In 1979, Peck showed that 13-*cis*-retinoic acid (RA) was an effective drug in severe acne in doses ranging from 1·0 to 3·3 mg/kg/bw, with a mean dose of 2·0 mg/kg/bw. Farrell, Strauss & Stranieri (1980), Jones *et al.* (1983a) and Plewig, Wagner & Braun-Falco (1980) have shown that 0·1, 0·5 and 1·0 mg/kg/bw are all equally effective, producing a 70% improvement over 12–16 weeks. Marsden *et al.* (1983) have reported a 50% improvement with 0·05 mg/kg/bw, and Jones, Greenwood & Cunliffe (1983b) have predicted that doses lower than this should be sebo-suppressive and be of benefit in patients with mild acne. Thus, there is a wide range of clinically effective doses of the drug, and the choice of dose will be influenced by other factors, namely toxicity and remission rates.

Most workers feel that the side-effects of the drug are dose-related, though only Plewig *et al.* (1980) and Jones *et al.* (1983a) have quoted actual incidences. Marsden *et al.* (1983) reported hyperlipidaemia with 0·05 mg/kg/bw and concluded that clinical effectiveness and toxicity are inseparable. The consensus therefore is that 13-*cis* RA should be given in short courses, and the determining factor for the dose becomes the remission period induced by it.

Peck *et al.* (1982), Strauss and Stranieri (1982) and Plewig, Nikolowski & Wolf (1982) have all reported long remission periods in their patients but have given either prolonged (6 months) or repeated courses of the drug until 100% clearance of the acne has been achieved. It is interesting to note that four out of five of Strauss's patients on 0·1 and 0·5 mg/kg/bw required a second course of the drug. Plewig also comments that patients in his 0·05–0·2 mg/kg/bw group tended to relapse.

We have approached the problem differently in that we have sought to establish the remission period induced by a single course (16 weeks) of 0·1, 0·5 and 1·0 mg/kg/bw of 13-*cis* RA. The effect of this therapy in these patients was reported previously (Jones *et al.*, 1983a). No difference was detected between the groups during therapy but the 0·1 mg dose did less well on initial follow-up. We have now followed these patients for up to 88 weeks post-therapy. In each treatment group, the patients have been divided into two categories so that the results could be analysed by life survival techniques (Peto *et al.*, 1977). The first category is defined as those patients who achieved clearance of their acne and required no further therapy during the follow-up period (i.e. 'remission'). The second category (i.e. 'relapse') required further therapy for a number of reasons: (i) whilst on treatment (a) failed to improve by 50% or (b) improved by 50% but had residual lesions; (ii) improved by greater than 50% but the acne relapsed post-therapy. All the patients were seen in Leeds for 28 weeks after discontinuing the drug, and were contacted by questionnaire 88 weeks post-therapy. Many of the patients had been referred by centres outside Yorkshire, and it was felt unfair to recall the patients unless they wished to see us for further advice. Sixty-nine out of seventy-six of the patients were accounted for by the questionnaire. The statistical analytical method chosen has the advantage of being able to incorporate the seven non-responders. The patients were simply asked whether the improvement in their acne had been maintained and whether they had required any therapy for their acne since last being seen.

From Table 1, it is apparent that the 0·1 mg/kg/bw dose has a considerably greater relapse rate than the other two doses, at both 28 and 88 weeks post-therapy. However, the log-rank significance test shows a statistically significant difference only at 88 weeks ($\chi^2 = 6{\cdot}2$, $P < 0{\cdot}05$). With doses of 0·5 and 1·0 mg/kg it appears that there is little change in the rate of remission between the 28th and 88th week, which suggests that if the patient's acne is relatively quiescent 6 months after stopping treatment then the prognosis is good. Patients on the larger doses and who remain in remission have a significantly slower return of their sebum excretion rate to their pretreatment values (Jones & Cunliffe, 1984). One feature that made this study difficult was the patients' tendency to demand treatment should there be a hint of recurrence but in only eight patients did the acne return to within 50% of their pretreatment level.

TABLE 1. Long-term results of 3 doses of 13-*cis*-retinoic acid in patients with severe acne

Dose (mg/kg/bw)	No. of patients	Patient categories	Weeks post-therapy 8	16	28	88
1·0	24	No therapy	23	20	15	14
		Further therapy[a]	1	4	9	10
		Relative relapse ratio[b]			0·72	0·69
0·5	30	No therapy	28	24	16	15
		Further therapy[a]	2	6	14	15
		Relative relapse ratio[b]			0·92	0·86
0·1	22	No therapy	19	12	8	5
		Further therapy[a]	3	10	14	17
		Relative relapse ratio[b]			1·53	1·66
		χ^2			3·65	6·240
		P			>0·1	<0·05

[a] Cumulative total.

[b] Peto (1977) p. 8, where relapse≡'death'.

We recommend the 0·5–1·0 mg/kg/bw dose as a balance between clinical effectiveness, toxicity and prolonged remission of the acne. Although 0·1 mg/kg/bw is effective in treating patients with severe resistant acne and is less toxic, it has a poor long-term effect and should not be used in such patients.

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Thalidomide or vitamin E therapy for discoid lupus erythematosus?

SIR, Knop *et al.* (1983) showed that in a group of sixty patients with chronic discoid lupus erythematosus treated with thalidomide, 90% showed complete or marked regression of the skin lesions. The lesions recurred when the treatment was discontinued, and repeated courses were needed to keep the eruption under control. They did not administer thalidomide to pregnant women because of its well-known teratogenic action, but other side-effects were frequently observed, including peripheral neuropathy, which in one patient lasted for a year after the medication was discontinued.

Vitamin E in a potent form, such as *d*, alpha-tocopheryl acetate or succinate in doses between 800 and 2000 i.u. daily has also given excellent results in discoid lupus erythematosus without side-effects. It may have to be continued indefinitely in a smaller maintenance dose, but the lack of side-effects renders this acceptable. Vitamin E improves the tone of the heart muscle and increases glycogen storage in the tissue, so patients with hypertension or a damaged heart, and diabetics on insulin should be started on small doses such as 100 i.u. once daily, which can be gradually increased while monitoring the blood pressure or the blood glucose.

Ayres and Mihan (1979) reported a group of seven patients with discoid LE who were treated with *d*, alpha-tocopheryl acetate, five of whom made an excellent recovery on doses ranging from 800 to 2000 i.u. daily. The two patients receiving only 300 i.u. showed a poor response, demonstrating the importance of an adequate dose. I have since successfully treated several additional cases of discoid LE and one patient with subacute disseminated LE with large doses of vitamin E.

Vitamin E protects cell membranes from lipid peroxidation, which releases enzymes which attach themselves to tissue proteins. The body may respond with the formation of autoimmune antibodies (Criep, 1969).

It would appear more logical in treating an autoimmune disease such as discoid lupus erythematosus, to protect cell membranes than to administer drugs such as thalidomide or immunosuppressive agents which can cause undesirable side-effects.

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SAMUEL AYRES, JR

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U.K. qualifying examination in dermatology

SIR, I am grateful for the interest in our proposals for a dermatological examination (*British Journal of Dermatology* 1984, **110**, 121–122) shown by such substantial 'establishment' figures as Janet Marks, Renwick Vickers and Sam Shuster (*British Journal of Dermatology* 1984, **110**, 736–737, 737–738, 738–739). If, like them, the majority of our colleagues are reasonably content with the present system of dermatological training, then the situation has been misjudged, but I am not convinced that this is so. Vickers clearly exposes the deficiencies of the existing arrangements, but vague assertions that they could be improved are unlikely to prompt any progress. It may be that there are better ways than a qualifying examination to enhance dermatological training, but we have not been able to think of one ourselves, and neither evidently has anyone else. I am well aware of the several practical difficulties enumerated by Marks and Shuster, but discussion of ways and means is surely taking the debate far beyond its present point. As Sam Shuster points out, there is in dermatology a lot of middle-aged flabbiness about. But willingness to accept the dermatological *status quo* without criticism or exploration of alternatives is hardly a testimony to drive, enthusiasm and vitality. Sam Shuster's response, like a raucous post-prandial burp, is quite good fun at the time but not likely to get us anywhere.

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M.W.GREAVES

Several other letters were received which made similar points (or similar noises) to those made by Drs Marks, Vickers and Shuster, but no other letters supporting the proposal for an examination were received. This correspondence is now closed. Editor.

British Journal of Dermatology (1984) **111**, 127.

Book Reviews

Photochemical and Photobiological Reviews. Volume 7. Edited by K.C.SMITH (1983) New York: Plenum Press. Pp. 373. Price $59.50.

This latest in the series, so well master-minded by Kendric Smith, has little of direct interest for dermatologists. The seven chapters include reviews of matters such as the electron spectroscopy of photoreceptors (i.e. target molecules), the remarkably radiation-resistant bacterium *Micrococcus radiodurans*, a fascinating concept called Cherenkov Radiation which is emitted as UV and visible radiation from matter bombarded with high frequency particles, and circadian rhythms in the filamentous fungus *Neurospora crassa*; you see what I mean. However, John Jagger gives a very good, and probably relevant account of UV-A effects on bacteria, Takashi Ito continues his explorations of phototoxicity, this time showing how it may help to elucidate the normal workings of the cell, and Thomas Moore reviews the relatively new technique of Photoacoustic Spectroscopy which does have dermatological applications for the study of keratin maturation, diffusion processes within the skin, sunscreen efficacy and skin lipid analysis.

B.E.JOHNSON

Manual of Dermatologic Therapeutics with Essentials of Diagnosis. Third Edition. K.A.ARNDT, M.D. (1983) Boston: Little, Brown and Co. Pp. 347. Price $16.95.

I was enthusiastic about the second edition of this handbook when I reviewed it in 1979. It is rather more than its name implies, as it does include sections on pathophysiology and diagnosis of the different common diseases with which it deals. It is purely factual, up-to-date and quite practical. It is a book I would whole-heartedly recommend to any General Practitioner working in isolation, and he would enjoy using it.

The major problem of a book of this nature, which became more evident on reading this new edition, is that it refers to North American trade names. I suspect this would discourage all but the keenest General Practitioner from buying it. The book has been translated into Spanish, Portuguese, Italian and Taiwanese, and I think that the time has come for a British English edition.

D.GOLDIN

Development of the Vascular System. Ciba Foundation Symposium 100 (1983) London: Pitman. Pp. 154. Price £25.

For anyone with an interest in blood vessels, especially angiogenesis, this book has considerable magnetism. The twenty-eight contributors from several disciplines including developmental biology, physiology, experimental pathology, vascular surgery, and yes, dermatology (T.J.Ryan) have made the proceedings such a good read, not solely by the quality of the formal contributions but more, I think, by the lively discussion which has also been published, and steered masterfully by the chairman, L.Wolpert.

This is not a heavy reference book but more a composition of fundamental problems in biology and pathology—with more questions than answers. What is an endothelial cell? Are the lining cells of lymphatic capillaries truly endothelial? Does the endothelium in microvasculature behave differently from that in large vessels? What is the role of heparin in angiogenesis? These and many other questions should stimulate dermatologists wishing to examine mechanisms in psoriasis, wound healing or tumour growth.

R.A.J.EADY

British Journal of Dermatology (1984) **111**, 128.

News and Notices

Congress of Aesthetic Medicine and Surgical Dermatology, Paris, September 1984

This Congress will be held at the Nikko Hotel, Paris, on 29 and 30 September 1984. For further information contact Dr J.J.Legrand, Société Français de Médecine Esthétique, 154, Rue Armand-Silvestre, 92400 Courbevoie, France.

The British Society for Dermatopathology, Keele University, September 1984

The British Society for Dermatopathology is holding a 1-day symposium entitled: 'Monoclonal Antibodies in Dermatopathology'. This will take place at Keele University, Staffordshire, U.K. on Wednesday, 19 September 1984 immediately before the winter meeting of the British Society of Investigative Dermatology. Guest speakers include Dr Kirk Ruiter from Leiden and Dr Julia Polak from London. For further details please apply to: Dr Robin Russell Jones, Secretary, British Society for Dermatopathology, St John's Hospital for Diseases of the Skin, Lisle Street, Leicester Square, London WC2H 7BJ, U.K.

International Course on Porphyrias and Porphyrins, Barcelona, February 1985

An International Course on Porphyrias and Porphyrins will be held in Barcelona, Spain (Department of Dermatology, Faculty of Medicine, Prof. J.M.Mascaro) on 14–15 February 1985. The lectures, in English, will be given by international specialists in this field. For further details write to: Dr M.Lecha, Department of Dermatology, Hospital Clinico & Faculty of Medicine, c/ Casanova 143, Barcelona-36, Spain.

International Symposium on Paediatric Dermatology, Trapani, September 1985

This will be held at Mazara del Vallo, Trapani, Italy on 18–21 September 1985. For further details contact G.Fabrizi, Department of Dermatology, Catholic University, 00168 Rome, Italy. (Tel. 06-33054227, 33054211.)

5th International Symposium on Bioengineering and the Skin

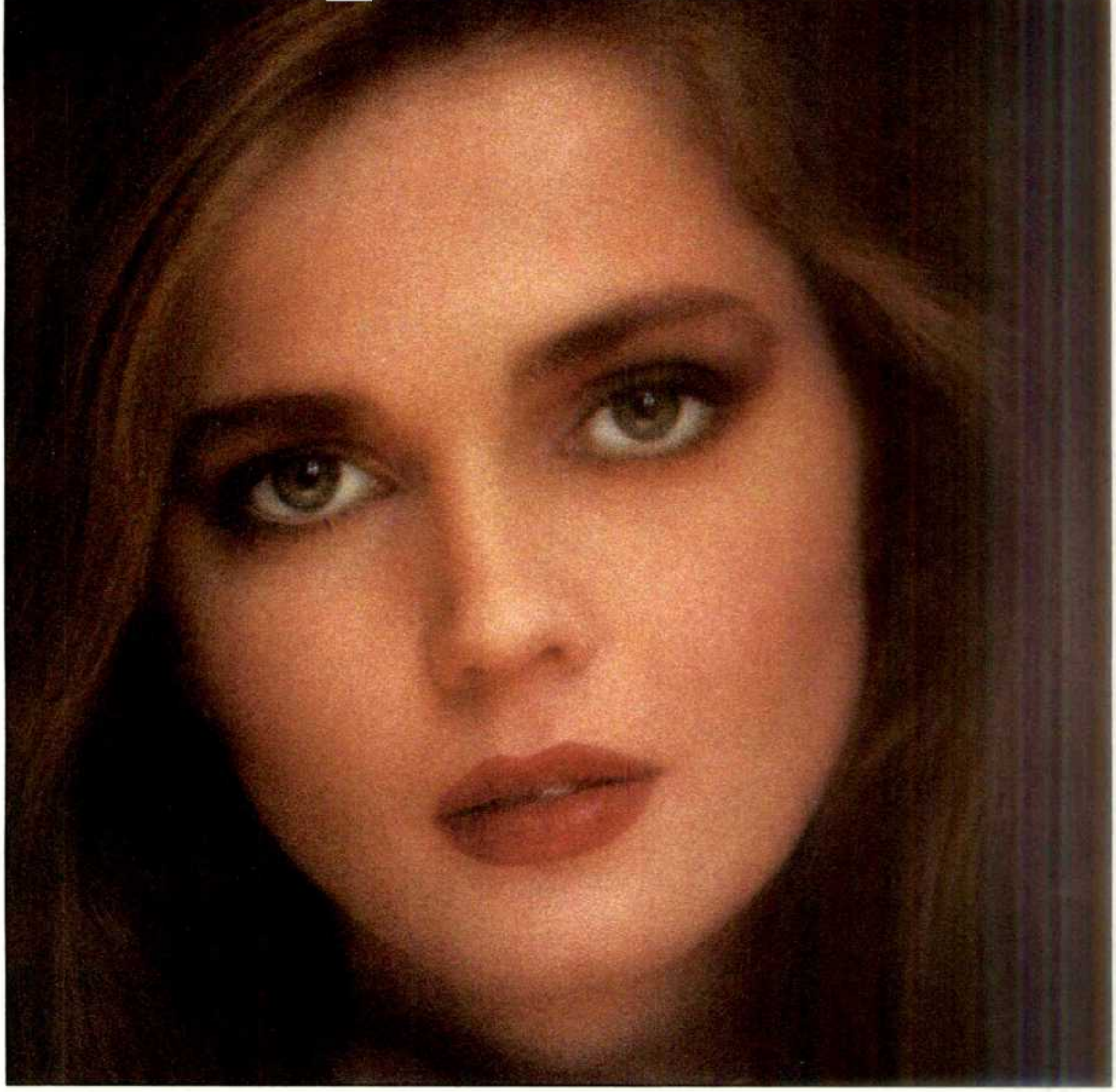
The International Society for Bioengineering and the Skin will be holding its 5th International Symposium in San Francisco from 29 August to 2 September 1985. The meeting will include paper presentations, workshops and poster sessions as well as the General Meeting of the Society. There will also be a 10-day basic introductory course in this field on the day prior to the meeting.

For further details and abstract forms please contact: Professor H.I.Maibach, Department of Dermatology, University of California School of Medicine, San Francisco, California 93143, U.S.A. or Professor R.Marks, Professor of Dermatology, Dept. of Medicine, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN, U.K.

International Seminar on Trans-Dermal Delivery Systems, San Francisco, February 1985

This course, primarily intended for scientists and others involved in the new area of trans-dermal delivery systems, will be held in San Francisco, U.S.A. from 1–3 February 1985. The presentations will emphasize practical approaches to the development and evaluation of trans-dermal systems. This meeting directly precedes the American Federation for Clinical Research in Carmel, California. For further information please contact: Howard Maibach, M.D., Department of Dermatology, University of California Medical School, San Francisco, California 94143, U.S.A.

THE ACCEPTABLE FACE OF COAL TAR



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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Manuscripts should be sent to the Editor, Dr J.L.Burton, Dermatology Department, Bristol Royal Infirmary, Bristol BS2 8HW. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. A duplicate copy, complete with figures, tables and references, should be sent, since in many cases two referees are consulted. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

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gram(s)	g	minute(s)	min	molar	mol l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3}g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hours(s)	h	milliequivalent	mmol		

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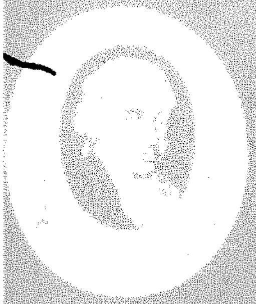

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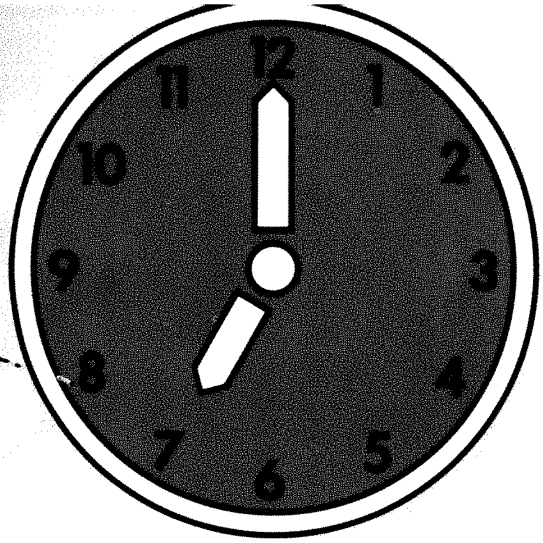

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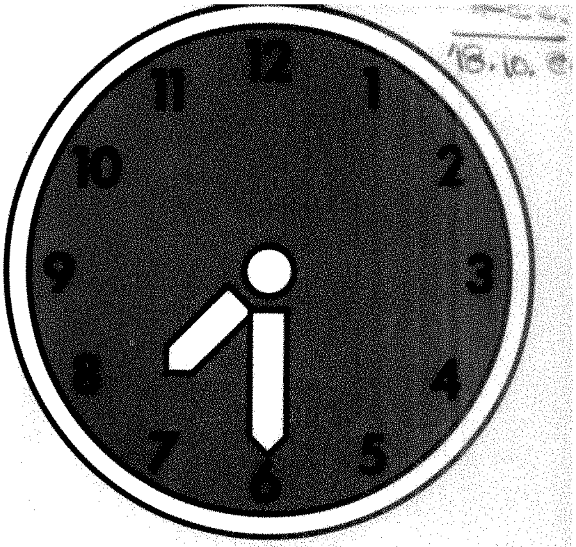

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Dithrocream 0.25%: 0.25% Dithranol BP.
Dithrocream Forte: 0.5% Dithranol BP
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INDICATIONS
For the topical treatment of sub-acute and chronic psoriasis including psoriasis of the scalp. Dithrocream Forte (0.5%) and Dithrocream HP (1.0%) should only be used once daily and for those patients who have failed to respond to lower strengths of dithranol. Dithrocream HP should normally only be used for short contact periods.

DIRECTIONS FOR USE
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CONTRA-INDICATIONS
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PRECAUTIONS
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PRODUCT LICENCE NOS
Dithrocream 0.1%: 0173/0029.
Dithrocream 0.25%: 0173/0028.
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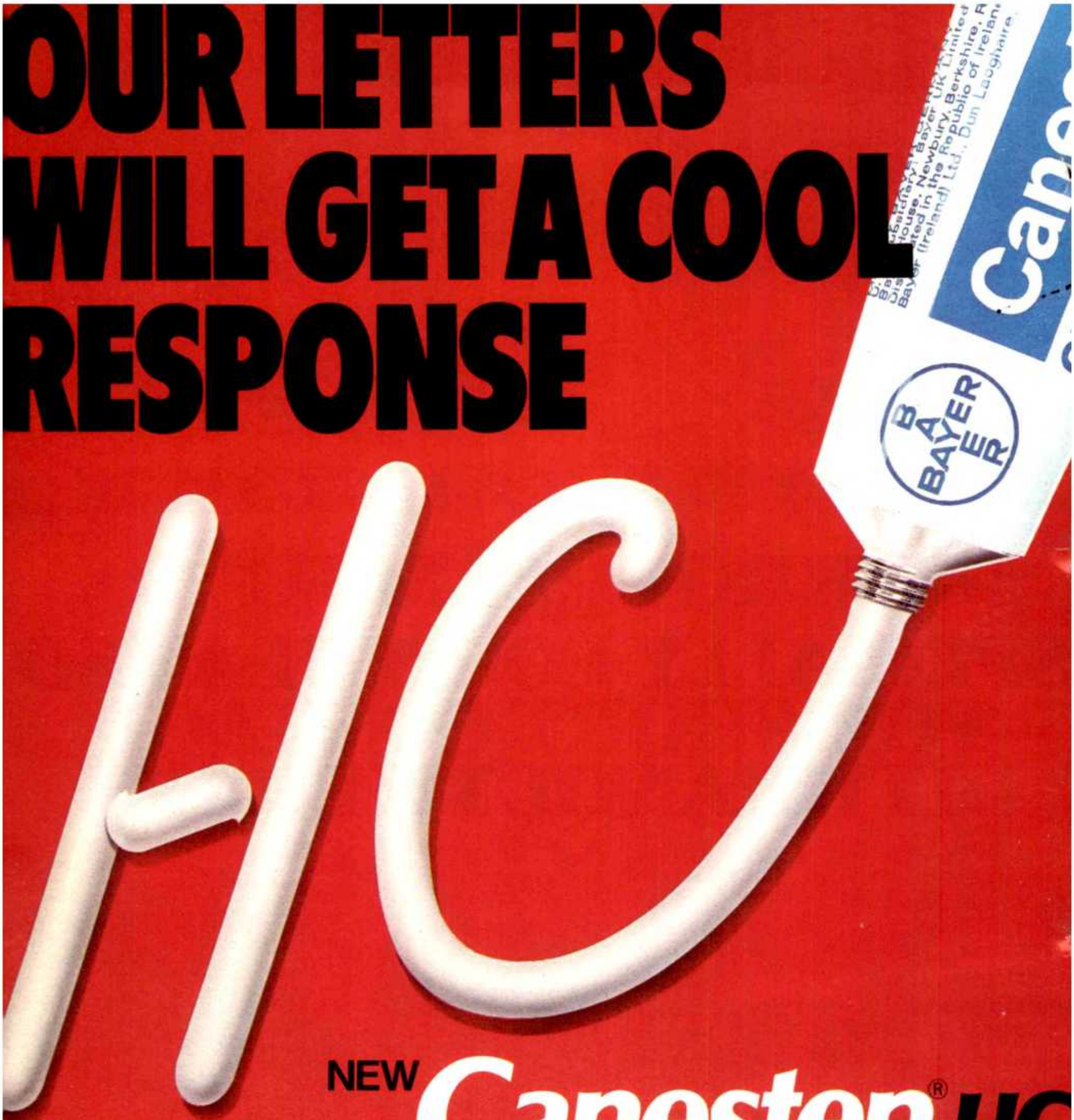

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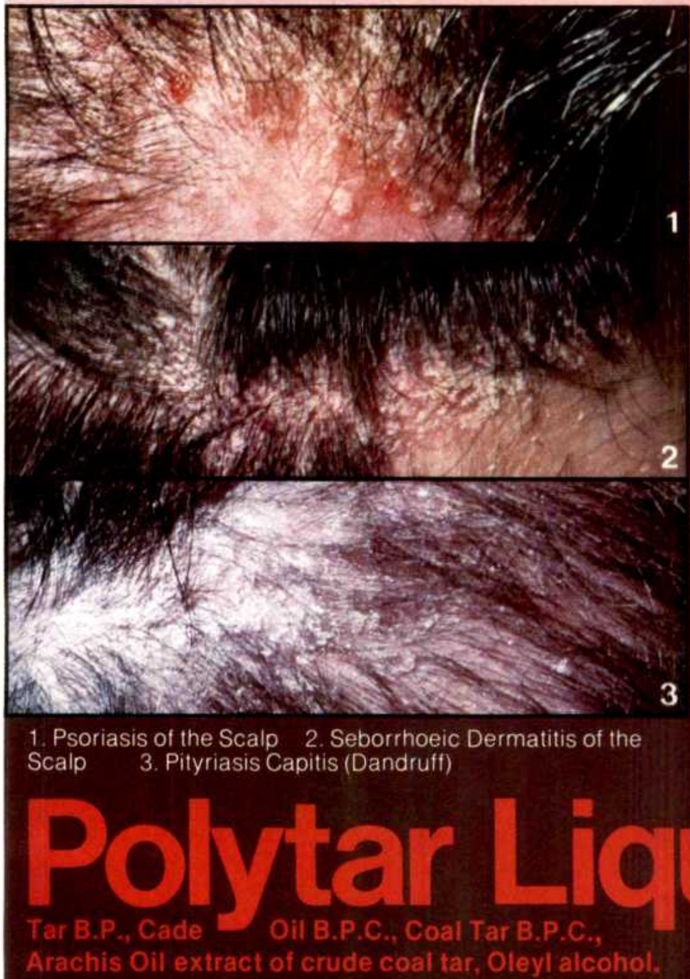
They all have one thing in common — the treatment



1. Psoriasis of the Scalp 2. Seborrhoeic Dermatitis of the Scalp 3. Pityriasis Capitis (Dandruff)

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Acknowledged as ideal for long-term control.

ALSO Polytar Plus with protein

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Tar B.P.	0.3%
Cade Oil B.P.C.	0.3%
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Arachis Oil extract of crude coal tar	0.3%
Oleyl alcohol	1.00%

Polytar Plus contains 3.0% hydrolysed animal protein, in addition to the above.

Uses Polytar Liquid and Polytar Plus are indicated in the treatment of scalp disorders such as psoriasis, seborrhoea, dandruff, eczema and pruritus. They are also of value in the removal of pastes and pomades used in the treatment of psoriasis.

Dosage and Administration The hair should be wetted and sufficient applied to produce an abundant lather. The scalp and adjacent areas should be vigorously massaged with the fingertips. The hair should then be thoroughly rinsed and the procedure repeated.
Polytar Liquid or Polytar Plus should be used once or twice weekly.

Contra-indications, warnings, etc. Nil

Pharmaceutical Precautions Store in a cool place

Packs and Prices

	Pack	Basic NHS price
Polytar Liquid	65ml	91p
	150ml	£1.34
	350ml	£2.37
	1 litre	£6.18
Polytar Plus	150ml	£1.43
	350ml	£2.80

Product Licence Number
Polytar Liquid PL0174/5016 Polytar Plus PL0174/0037

Full information and Data sheet on request.

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Dosage and administration: Four times a day, moisten brush with Monphytol and apply to the affected parts, reaching gently into the folds of the skin. Treatment should be repeated from time to time after the condition has subsided to prevent reinfection.

Contra-indications, warnings, etc: Monphytol may sting sensitive weeping areas of acutely inflamed skin. Other treatment (to reduce inflammation and exudation) may first be necessary. Although there is only a small amount of boric acid present, caution is advised in the repeated application of Monphytol to extensive areas of the skin.

Pharmaceutical precautions: Store away from heat.

Legal category: P.

Package quantities: Bottles containing 18 ml with brush fitted in cap.

Further information: Nil.

Product licence number: 0118/5010.

Basic NHS price: £0.57 per 18 ml bottle.

Reference:

1. Krebs HA, Wiggins D, Stubbs M, Sols A, Bedoya F. Studies on the mechanism of the anti-fungal action of benzoate. Biochem J. 1983; **214:** 657-63.

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Dosage Adults and children over 12 years. One tablet twice daily. Minocin may be taken with meals. Treatment of acne should be continued for a minimum of six weeks. **Contra-indications** Known tetracycline hypersensitivity. Systemic lupus erythematosus. **Precautions** Absorption is impaired by iron and antacids containing calcium, magnesium and aluminium salts. **Warnings and side effects** Gastro-intestinal disturbances are the side effects most commonly reported. A yellow to brown discoloration of teeth is a possible sequela of treatment during pregnancy or early childhood (up to 8 years of age). **Presentation** Beige tablets containing 50mg of minocycline as the hydrochloride, supplied in bottles of 100 at a basic NHS cost of £22.44 . PL/0095/0062. MINOCIN is a trademark. Full prescribing information is available on request.

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For an international view of dermatology practice . . .
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JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY

OFFICIAL PUBLICATION: American Academy of Dermatology
ESTABLISHED: July, 1979 **PUBLISHED:** Monthly **EDITOR:** J. Graham Smith, Jr., M.D.

The **JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY** is a professional journal devoted exclusively to the clinical and continuing education needs of the entire dermatologic community, including those family practitioners, pediatricians, and internists who are concerned with clinical manifestations of skin disease in their practice.

The journal serves as the official publication of the 6,300 member American Academy of Dermatology, the largest society in the specialty.

The 140 text pages published each month include original scientific contributions describing clinical studies. Other sections include Continuing Medical Education (CME) articles based on the Core Curriculum of Clinical Dermatology developed by the American Academy of Dermatology; Clinical review articles; Therapy articles; Clinical and laboratory studies; Gross and microscopic symposium; Editorials; Commentary; Meeting reports; Reports from other fields and Book reviews.

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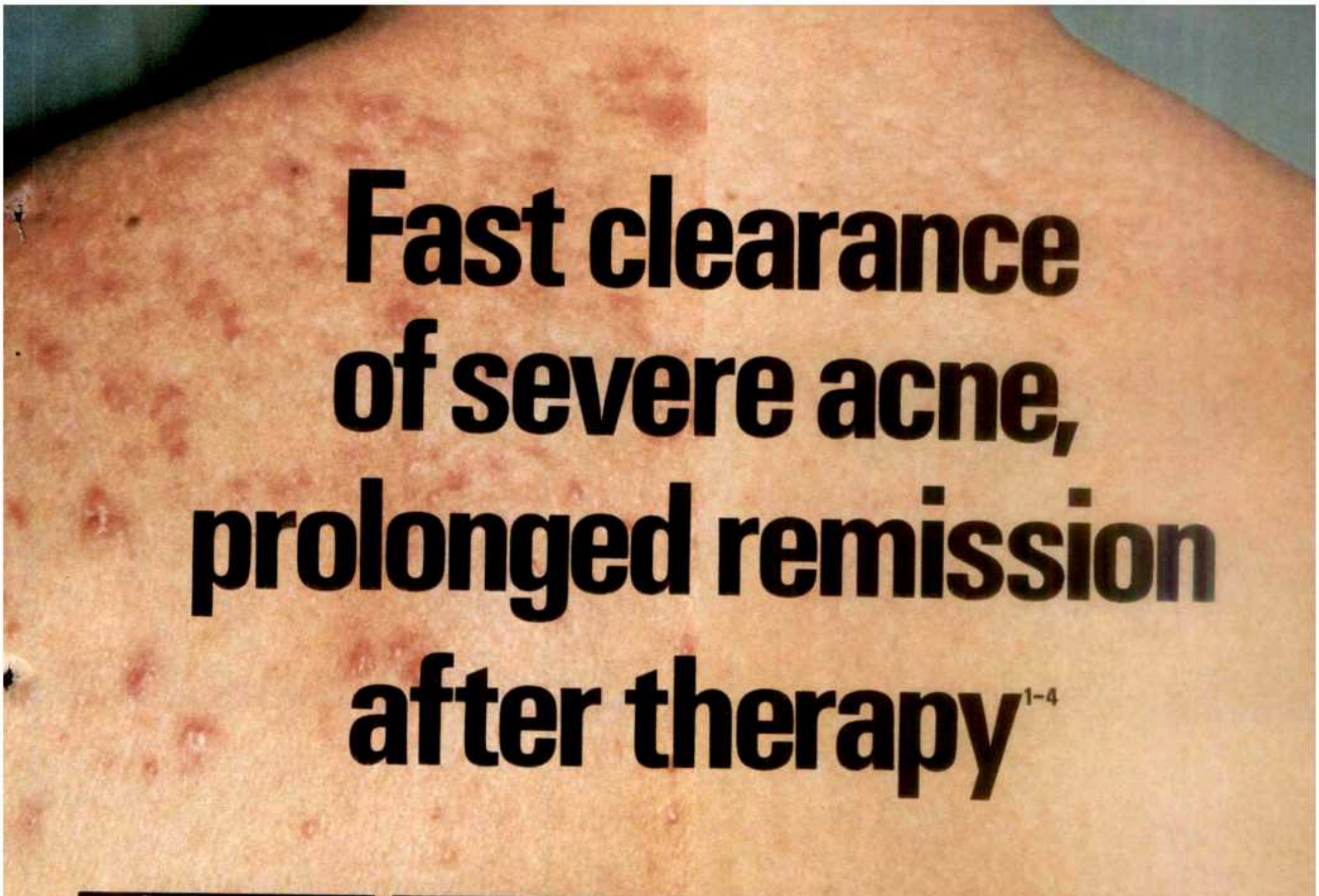
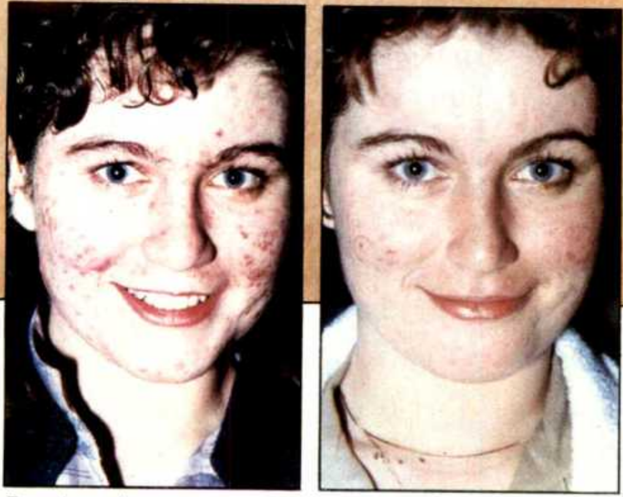


Fast clearance of severe acne, prolonged remission after therapy[1-4]



Female patient unresponsive to oral antibiotics, before and after a course of Roaccutane.

"The aim of therapy is not only to produce clearing of active disease, but also to maintain this clearing indefinitely."[5]

In severe acne, Roaccutane rapidly produces an excellent response in 90% of patients and prolonged remissions after therapy once clearance is achieved.[1-4]

(Main picture) Male patient with truncal acne unresponsive oral antibiotics, before and after a course of Roaccutane.

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a turning point in acne therapy, for both male and female patients

BRIEF PRESCRIBING INFORMATION

Indications Cystic and conglobate acne, severe acne unresponsive to systemic antibiotics.

Dosage 0.5mg/kg daily for 12-16 weeks. Increase up to 1mg/kg daily if response is poor at 4 weeks. Reduce dosage if tolerance is poor. As a rule, other anti-acne therapy, including antibiotics, is not indicated. Repeat course of treatment should not normally be given.

Contra-indications Pregnancy (Roaccutane is teratogenic—see 'Precautions') and breast feeding. Hepatic or renal impairment.

Precautions The following precautions must be observed in women of childbearing potential.

1. Patients should be given detailed explanation of the risks.
2. Pregnancy should be excluded before therapy is instituted.
3. Effective contraception must be ensured for one month before treatment, during treatment, and for one month after treatment ends.

Use of patient information leaflets is strongly recommended. Monitor liver function and serum lipids (fasting value) regularly.

Side-effects Mainly mucosal dryness and fissures, facial dermatitis, desquamation, epidermal fragility, conjunctivitis, arthralgia/myalgia, increased hair loss (uncommon and reversible), possible elevation of liver enzymes and serum triglycerides. Benign intracranial hypertension has been reported, particularly in association with concomitant antibiotics.

Availability Hospitals and hospital clinics.

Presentations and Basic NHS Cost

Roaccutane capsules 5mg × 100 £33.56

Roaccutane capsules 20mg × 100 £96.73

Product Licence Numbers 0031/0158 (capsules 5mg), 0031/0160 (capsules 20mg).

Licence Holder

Roche Products Limited
PO Box 8, Welwyn Garden City
Hertfordshire AL7 3AY

References

1. In "Retinoids: Advances in Basic Research and Therapy" Eds. Orfanos, C.E. *et al.* Springer-Verlag, Berlin 1981, p.219
2. Brit. J. Derm., 1983, *108*, 333
3. J. Amer. Acad. Derm., 1982, *6*, 735
4. J. Amer. Acad. Derm., 1982, *6*, 766
5. J. Amer. Acad. Derm., 1983, *9*, 629

Roaccutane is a trade mark.

ROCHE Retinoids **From Roche—world leader in retinoid research**

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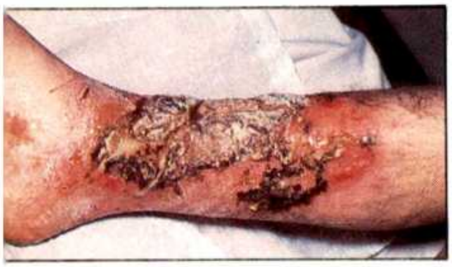
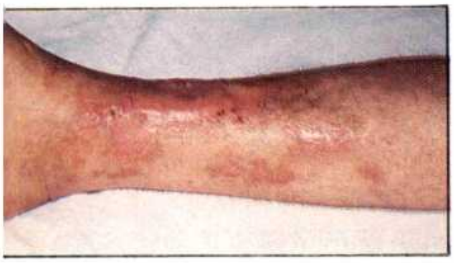


7 year old heroin addict with
xtensive infected ulceration.



months later after treatment with
)DOSORB and oral erythromycin.

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RESCRIBING INFORMATION

resentation: Sachets of sterile cadexomer iodine, a dry yellow-brown powder of odified starch gel microbeads containing iodine Ph.Eur. at a concentration of 9% w/w for topical application. **Uses:** Treatment for chronic leg ulcers sociated with venous disease. Treatment of pressure sores. **Dosage & dministration:** 'Iodosorb' is applied to the wound surface to a minimum depth 3mm and covered with a dry, sterile non-adherent dressing. The dressing ould be changed daily or when the 'Iodosorb' has become saturated with ound exudate. Each time the dressing is changed and at the end of treatment, e remaining 'Iodosorb' should be gently washed from the ulcer surface either ith a stream of sterile water or with a wet sterile swab. To avoid the risk of cross-contamination, it is recommended that a sachet of 'Iodosorb' be confined to the treatment of a single patient per day. 'Iodosorb' is a topical treatment for the wound and has no influence on the underlying disturbance of circulatory function. Where considered appropriate, support bandages or stockings can be applied in conjunction with the use of 'Iodosorb'. **Contra-Indications, Warnings etc: Contra-Indications:** As 'Iodosorb' contains 0.9% iodine, it should not be used in patients with known or suspected iodine sensitivity. **Warnings:** Iodine is absorbed systemically especially when large ulcers are treated. The use of 'Iodosorb' should be avoided in pregnant or lactating women; absorbed iodine can cross the placental barrier and is secreted into breast-milk. **Precautions:**

In wet, venous leg ulcers

IODOSORB

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fast, active healing

Systemic absorption of iodine should be taken into consideration if the thyroid function of a patient is under investigation. It has been observed occasionally that an adherent crust can form when the dressing is not changed with sufficient frequency. These crusts can be softened during dressing changes. **Side-Effects:** Some patients experience a transient smarting sensation during the first hour after application of 'Iodosorb'. Mild erythema without sensitisation has also been reported. **Pack Size & Basic NHS Price:** Box of 7 x 3g sachets. £10.95. **Product Licence Number:** 29/0165. **References:** 1. Ormiston M.C., *et al*, in Fischer H. eds Cadexomer Iodine. Stuttgart: F. K. Schattauer. 1983:63. 2. Moberg, S., *et al*, J. Amer.Geriat.Soc.1983; 31:462. 3. Skog, E., *et al*, Br. J. Dermatol 1983; 109:77.

Further information available on application to the company:
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'Iodosorb' is a trade mark

'Iodosorb' is a trade mark

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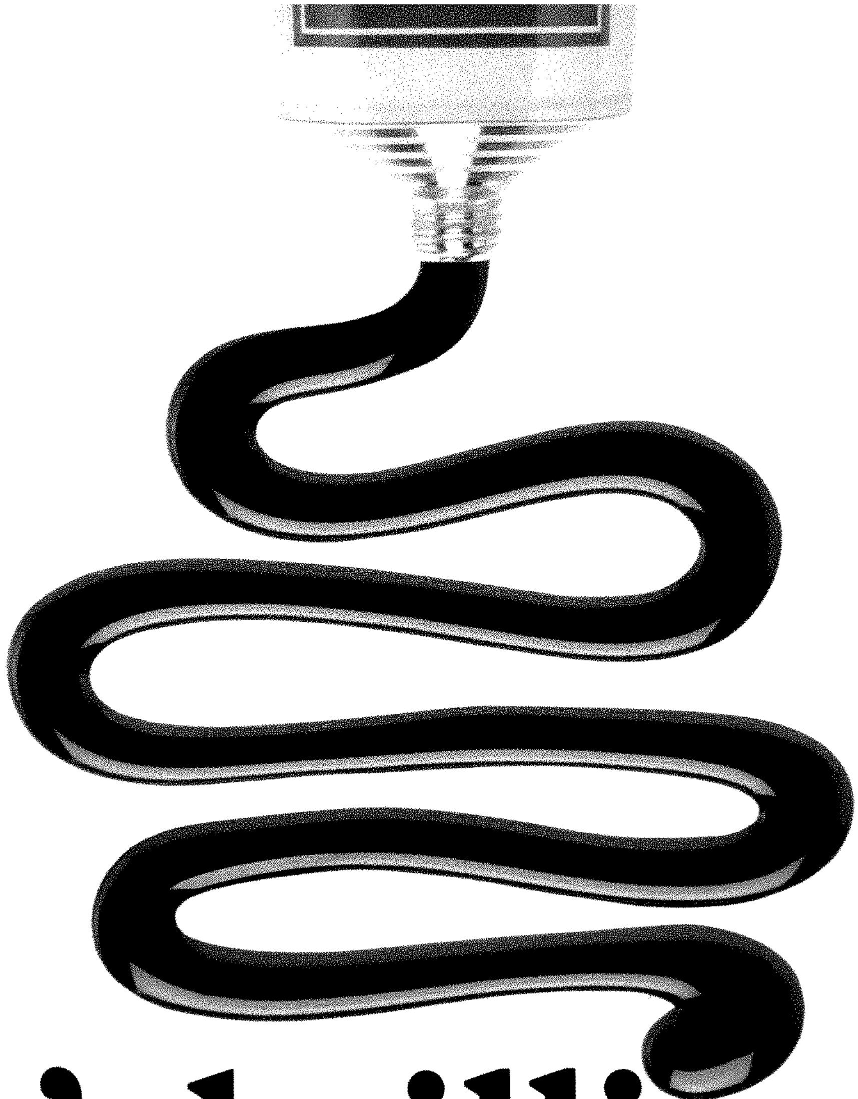

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Prescribing Information
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Directions: Clean and dry the ulcer site, apply and smooth the Variclene gel over the affected area. Cover with a sterile dressing or pad and, where appropriate, apply compression bandaging. Repeat as required at intervals of not more than seven days.
Contra-indications, Warnings, etc: Avoid contact with clothing and linen. Occasionally, transient stinging on application may occur. In cases of arterial or extensive, painful venous ulceration, this may be prolonged, when treatment should be discontinued until greater tolerance has been achieved. Keep away from the eyes.
Presentation: 50g tube **Product Licence:** 0173/0015 (PA 278/10/1)

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Green aqueous gel containing 0.5% w/w Brilliant Green BP, 0.5% w/w Lactic Acid BP.

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British Journal of Dermatology (1984) **111**, 129–138.

Clinical and Laboratory Investigations

Prognostic classification of malignant melanoma by clinical criteria

W.FUNK*, CH.SCHMOECKEL*, D.HÖLZEL† AND O.BRAUN-FALCO*

*Department of Dermatology, University of Munich and †Department of Medical Statistics, University of Munich, Munchen, West Germany

Accepted for publication 30 January 1984

SUMMARY

In a retrospective study of 503 well-documented cases of primary malignant melanoma (stage I) clinical criteria were analysed for their prognostic relevance. The maximum elevation (in mm) of the tumour was found to be the most important single prognostic factor. There was a close association with tumour thickness, measured histologically by the method of Breslow (correlation coefficient = 0·73). A combination of elevation and three additional clinical criteria (site, nodule- or lesion-diameter, and surface defects such as erosion, ulceration or bleeding) allowed a further improvement in prognostic accuracy. This clinical classification into low-risk and high-risk melanomas was as effective as the use of tumour thickness measured histologically, and can therefore be used for the preoperative planning of treatment.

Many studies of malignant melanoma have been published in which prognostic criteria were analysed with particular regard to histological features, such as level of invasion (Clark *et al.*, 1969), tumour thickness (Breslow, 1975), mitotic index (Schmoeckel & Braun-Falco, 1978) and ulceration (Balch *et al.*, 1980). However, little information is available as to whether the determination of prognosis is possible by using only clinical criteria. A clinical assessment of the prognosis for individual melanoma patients could be helpful in deciding preoperatively the optimum treatment. For low-risk melanomas, where an exact diagnosis of malignant melanoma using frozen sections can be difficult, a simple excision biopsy might be sufficient; in these cases the histological diagnosis can be awaited and further therapy is needed only if a worse prognosis is predicted. For medium-risk or high-risk cases an elective *en bloc* node dissection can be chosen. If preoperative irradiation is thought to be helpful, it might be restricted to medium-risk or high-risk cases only.

In two previous retrospective studies, both the histological and the major clinical criteria were investigated by means of uni- and multivariate analyses (Schmoeckel *et al.*, 1983a, b). In another study the prognostic significance of the resection margin was examined (Schmoeckel *et al.*,

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Correspondence: Ch. Schmoeckel, M.D., Dept. of Dermatology, University of Munich, Frauenlobstr. 9–11, D–8000 München 2, West Germany.

1983c). In this present paper, 503 well-documented cases of primary malignant melanoma (stage I) were analysed in different ways to determine the most effective clinical prognostic criteria, considering both the patient (age, sex, site) and the tumour (horizontal size, elevation, shape, ulceration, bleeding, etc.). The results were also compared with histopathological criteria.

METHODS

During the years 1962 to 1972, 2242 cases of primary cutaneous malignant melanoma were collected in the 'German Melanoma Study Group'. Of these, 503 stage I cases meeting the following criteria were studied.

(a) *Follow-up*. 264 cases (52·5%) were disease-free for at least 5 years (with an average follow-up of more than 9 years); 239 patients (47·5%) later had histologically proven metastases.

(b) *Treatment*. All patients were treated surgically. Although resection margins varied considerably (from 1 to 50 mm), an influence on prognosis could be excluded by single factor and multivariate analysis. Previous radiotherapy, prophylactic lymph node clearance, adjuvant immunotherapy or chemotherapy were not performed.

(c) *Histology*. In all cases histological slides were available. In most cases several sections were examined, but serial or step sections were not made. Cases in which the sections appeared not to represent the centre of the tumour were discarded.

(d) *Photodocumentation and medical records*. In all cases preoperative black-and-white photographs (oblique-view pictures with a millimetre scale) and extensive clinical documentation were available. Cases with poor photographs or limited documentation were discarded.

The following criteria were computerized.

Clinical criteria

These were determined by the clinical documentation and by photographs.

(1) *Age* (at time of surgical treatment).

(3) *Location of the tumour*.

(4) *Composition of the tumour:* flat areas and/or numbers of nodules.

(5) *Satellite metastases*. Distance to the primary, number, diameter and elevation were recorded.

(6) *Horizontal diameters* (length, width) of the tumour. Length and width of the nodule (most elevated structure of the tumour) were recorded separately.

(7) *Maximum elevation of the tumour*. Maximum height of the tumour above skin surface level (in mm) was estimated from black-and-white photographs (oblique-view pictures with a millimetre scale) and from descriptions in the records.

(8) *Profile of the nodule:* (a) plateau-like; (b) convex; (c) transitional; (d) polypoidal. We also determined whether it was polynodulated (nodules merging into each other) or not.

(9) *Colour*. Areas of regression and amelanotic areas were recorded.

(10) *Surface alterations:* (a) smooth; (b) porous; (c) papular; (d) verrucous.

(11) *Surface defects:* (a) erosion or ulceration as indicated by the patient; (b) erosion or ulceration as seen clinically; (c) bleeding as seen clinically or as indicated by the patient.

Histological criteria

(12) *Tumour type* according to Clark *et al.* (1969).

(13) *Level of invasion* according to Clark *et al.* (1969).

(14) *Tumour thickness* according to Breslow (1975).

(15) *Mitotic index* according to Schmoeckel & Braun-Falco (1978) defined as the number of mitoses/mm^2.

(16) *Prognostic index* according to Schmoeckel & Braun-Falco (1978) defined as the product of tumour thickness and mitotic index.

Statistical methods

(a) *Single factor analysis.* Normally the chi-square test was used. In cases with too small numbers, the exact Fisher test was used (significant if $P \leqslant 0{\cdot}01$). Correlation coefficients were determined from among various criteria ($P \leqslant 0{\cdot}01$).

TABLE 1. Results of single factor analysis and stepwise logistic regression analysis (dependent variable: later metastasis—no or yes); the *P* values are shown for different clinical criteria

Criteria *n* = 503	Number of cases ('no'; 'yes') or range	Single factor analysis *P*	Stepwise logistic regression *P*
(1) Age	19–85 years	0·2624	—
(2) Sex	♀345; ♂158	0·0000	—
(3) Location	see Fig. 2*	0·0000	0·0001
(4) More than one individual nodule	456; 47	0·0408	0·0676
(5) Satellite	470; 33	0·0083	0·0492
(6) Maximum diameter (length)			
of the total lesion	3–75 mm	0·0000	—
of the nodule	2–55 mm	0·0000	0·0077
(7) Maximum elevation	0–20 mm	0·0000	0·0000
(8) Profile of the nodule			
plateau-like	476; 27	0·0068	0·0358
convex	232; 271	0·3018	n.t.
transitional	422; 81	0·0000	—
polypoid	450; 53	0·0000	—
polynodulated	448; 55	0·0000	—
(9) Colour			
regression	437; 66	0·5326	n.t.
amelanotic part	420; 83	0·0394	—
(10) Surface alterations	177; 72; 214; 40*	0·0006	—
(11) Surface defects (all kinds)	187; 316	0·0000	0·0017
erosion or ulceration (as seen clinically)	366; 137	0·0000	—
a history of erosion or ulcer	292; 211	0·0000	n.t.
bleeding	222; 281	0·0000	—

* Subgroups as described in Methods.

n.t. = not tested in the regression analysis.

—: not accepted in the regression analysis or excluded again (without additional prognostic information), $P \geqslant 0{\cdot}1$.

(b) *Multivariate analysis.* We used the stepwise logistic regression model (condition for acceptance or exclusion: $P<0{\cdot}1$ or $P>0{\cdot}15$) which was derived from *BMDP Statistical Software* (University of California Press, Berkeley, 1981).

(c) *Qualitative and quantitative aspects in prognosis.* The following conditions (qualitative aspect) appeared to be justified: a low-risk group should have less than 10% of metastatic cases and a high-risk group should have more than 70% of metastatic cases. Furthermore, low-risk and high-risk groups should contain as many patients as possible (quantitative aspect).

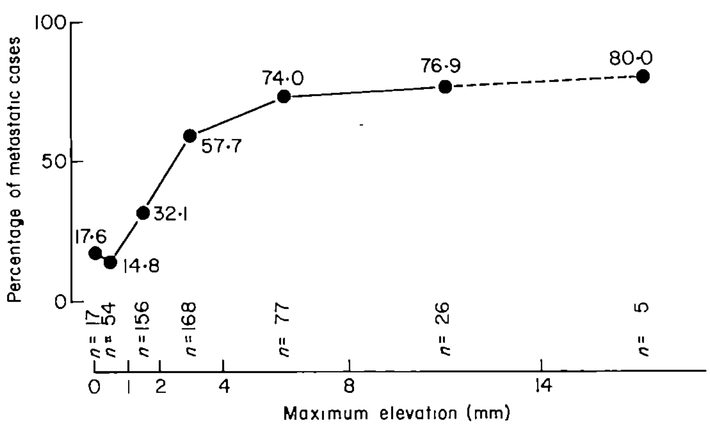

FIGURE 1. Maximum elevation (in mm) and percentage of metastatic cases (the four prognostically relevant classes are shown in Fig. 4).

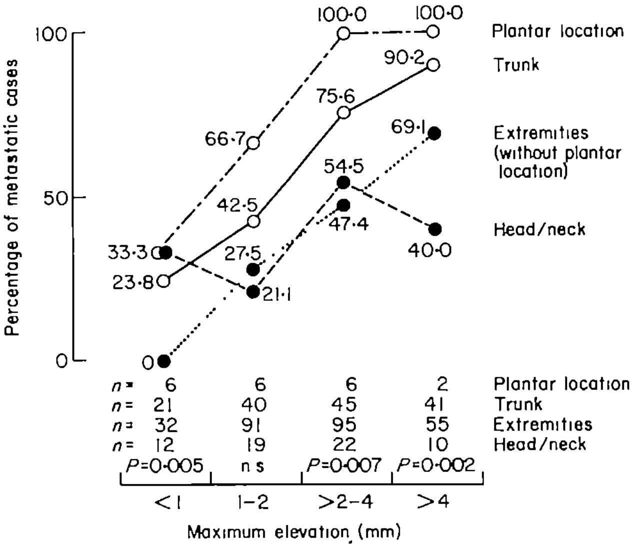

FIGURE 2. Location and percentage of metastatic cases on different maximum elevation levels Plantar tumours were excluded from statistical analysis due to their low frequency.

TABLE 2. Maximum diameter (horizontal length) and percentage of metastatic cases on different maximum elevation levels (the total number of cases is given in parentheses)

	Maximum elevation (mm)			
Diameter (mm)	< 1	1–2	> 2–4	> 4
Flat tumours (total lesion)				
< 15	6·5% (31)			
15–19	16·7% (12)			
20–24	18·2% (11)			
≥ 25	29·4% (17)			
Elevated tumours (nodule)				
< 5		16·7% (18)	25·0% (4)	0
5–9		24·1% (54)	51·8% (56)	71·4% (7)
10–14		40·0% (45)	52·0% (75)	75·9% (29)
≥ 15		41·0% (39)	84·8% (33)	75·0% (72)

RESULTS

Prognostically relevant criteria

(1) *Maximum elevation* (in mm) appeared to be the best prognostic indicator; the P value was lowest in the stepwise logistic regression analysis (Table 1, Fig. 1).

(2) *Location of the tumour* was an important prognostic factor even with different maximum elevation levels (Fig. 2, Table 1).

(3) *Satellites* were a bad prognostic sign (Table 1), particularly if more than 1 cm from the primary (100% metastatic cases when > 1 cm, $n = 6$; only 63·0% when ≤ 1 cm, $n = 27$).

(4) *Maximum diameter* (horizontal length) of the total lesion for flat tumours (maximum elevation < 1 mm) and of the nodule in distinctly (≥ 1 mm) elevated tumours (Tables 1 and 2). If maximum elevation was more than 4 mm, neither the diameter of the total lesion nor of the nodule had any prognostic relevance (Table 2).

(5) *Surface defects* such as erosion, ulceration, or a history of bleeding, especially for more

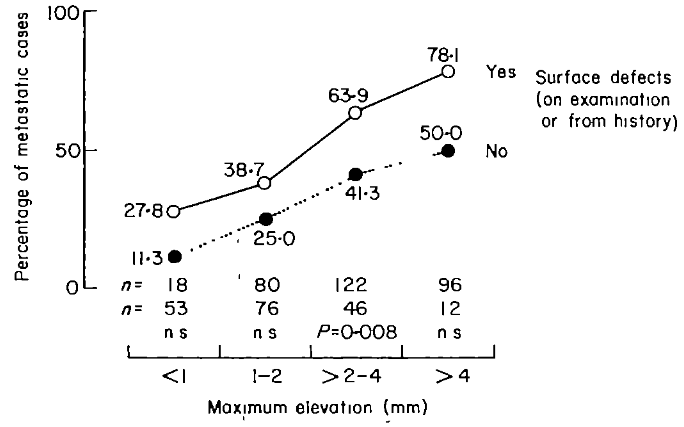

FIGURE 3. Surface defects (erosion, ulceration, bleeding) and percentage of metastatic cases on different maximum elevation levels.

TABLE 3. Prognostic classification of primary malignant melanoma (stage I) using clinical criteria

Low-risk melanomas (recurrences in 3%)	High-risk melanomas (recurrences in 80%)
Maximum elevation < 1 mm without erosion, ulceration, bleeding or satellite Except in cases with both factors: (1) unfavourable site: head/neck, trunk, plantar (2) diameter (total lesion) ≥ 15 mm	Maximum elevation > 2–4 mm and at least two risk factors: (1) trunk or plantar location (2) diameter (nodule) ≥ 15 mm (3) erosion/ulceration/bleeding OR Maximum elevation > 4 mm and at least one risk factor· (1) trunk or plantar location (2) erosion/ulceration/bleeding

elevated tumours (Fig. 3, Table 1). The kind of surface defects (erosion/ulceration or bleeding, which was in most cases only a history of bleeding) seemed to be of less importance (Table 1).

(6, 7) Two more criteria, the ***plateau-like lesion*** (favourable sign; it was seen only with an elevation of 1–2 mm) and ***more than one individual nodule*** (unfavourable sign) were accepted into the regression model (Table 1).

Although most of the other criteria were significantly connected with the frequency of later metastases, if used alone they did not improve the accuracy of prognostication (Table 1).

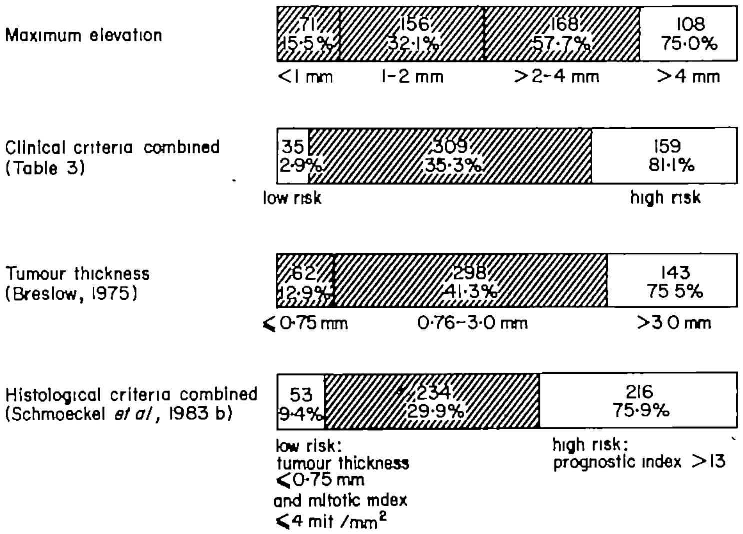

FIGURE 4. Prognosis in 503 cases: qualitative and quantitative comparison of maximum elevation, clinical criteria combined, tumour thickness (Breslow, 1975) and histological criteria combined (Schmoeckel *et al.*, 1983b). The number of cases and the percentage of metastatic cases are given. Cases neither low-risk nor high-risk are hatched.

TABLE 4. Results of stepwise logistic regression analysis (dependent variable: later metastasis—no or yes) of the prognostically relevant clinical criteria (Table 1 'stepwise logistic regression') and sex and age as well as of histopathological criteria (482 cases with complete data)

Criteria $n=482$	Single factor analysis P	Stepwise logistic regression P
Clinical criteria		
(1) Age	0·2191	—
(2) Sex	0·0000	—
(3) Location	0·0001	0·0063
(4) More than one individual nodule	0·0101	0·0550
(5) Satellite	0·0137	0·0921
(6) Maximum diameter of the nodule	0·0000	0·0076
(7) Maximum elevation	0·0000	0·0003
(8) Profile of the nodule: plateau-like	0·0063	0·0335
(11) Surface defects (all kinds of)	0·0000	0·0129
Histopathological criteria		
(12) Tumour type	0·0003	—
(13) Level of invasion	0·0000	—
(14) Tumour thickness	0·0000	—
(15) Mitotic index	0·0000	0
(16) Prognostic index	0·0000	n.t.

n.t. = not tested in the regression analysis.

—: not accepted in the regression analysis or excluded again (without additional prognostic information), $P \geqslant 0{\cdot}1$.

Combination of criteria for a prognostic classification

A prognostic classification (considering both qualitative and quantitative aspects) based upon maximum elevation and taking into account further criteria is shown in Table 3. Cases with head/neck location and a large diameter ($\geqslant 15$ mm) were excluded from the low-risk group because of a relatively high metastatic rate (42·9%; see also Fig. 2); however, this finding is of limited significance due to the small number of cases ($n=7$). Using the other criteria 'plateau-like lesion', 'satellite' and 'more than one individual nodule' only a few more low- and high-risk cases were identified; also these criteria had relatively high P values in the regression analysis (Table 1). The combination of clinical criteria (Table 3) was more prognostically effective than maximum elevation alone (Fig. 4): only then could low-risk cases be identified. In this way the number of high-risk cases was also increased.

Comparison of clinical and histopathological criteria

A further multivariate analysis using both clinical and histological criteria ($n=482$) revealed the significance of all seven clinical criteria accepted in the regression analysis in Table 1 by also appearing in the stepwise logistic regression analysis (Table 4). Additional prognostic information was obtained only by means of the mitotic index, whereas level of invasion and

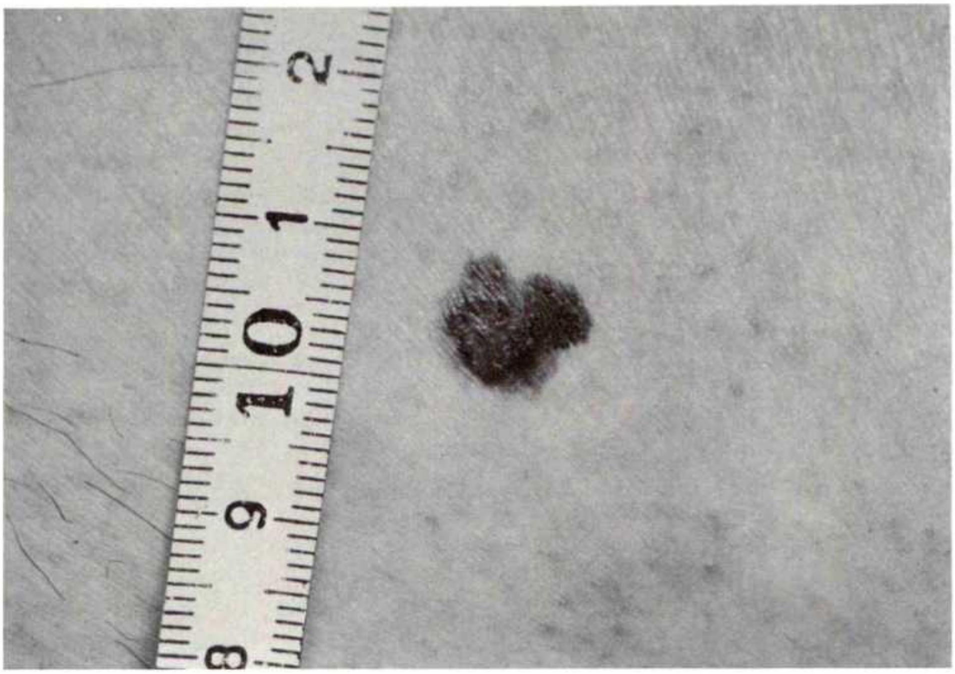

FIGURE 5. Low risk malignant melanoma: maximum elevation < 1 mm; location on the trunk but horizontal diameter < 15 mm.

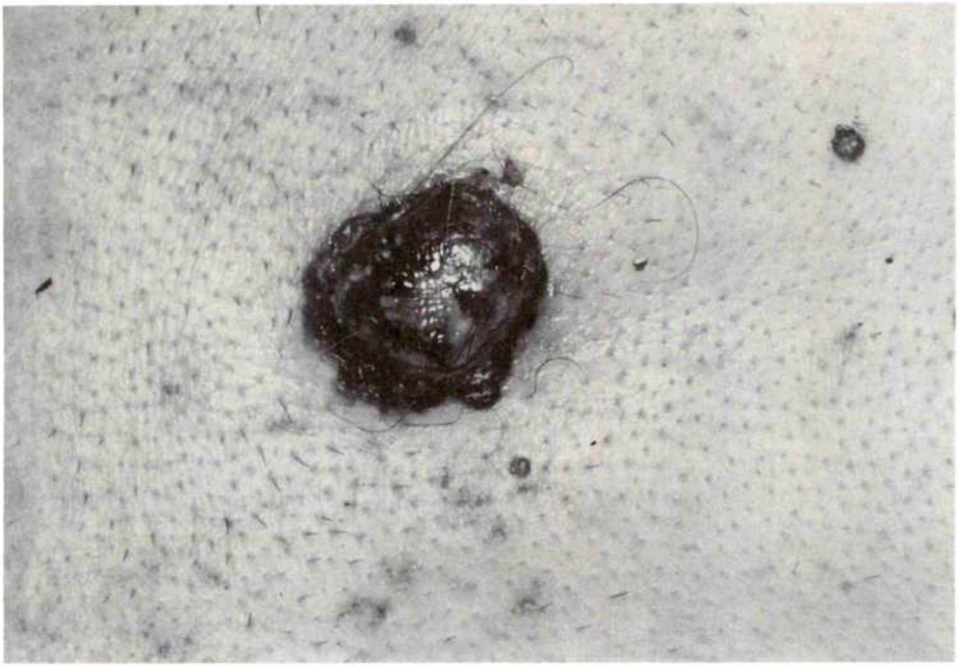

FIGURE 6. High risk malignant melanoma: maximum elevation high (> 4 mm) with one risk factor (ulceration).

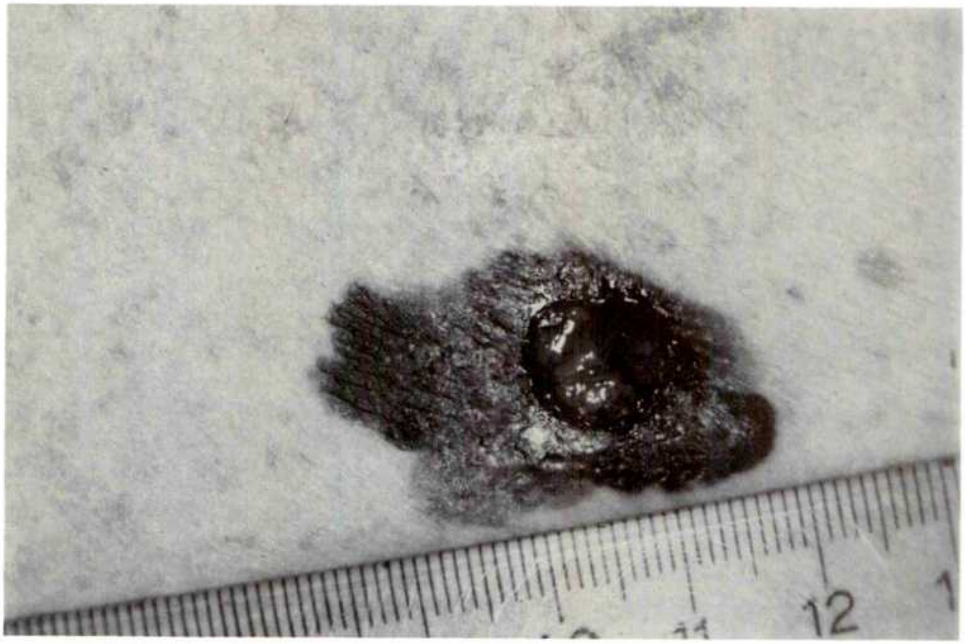

FIGURE 7. High risk malignant melanoma: maximum elevation intermediate (2–4 mm) with two risk factors (ulceration and trunk location).

tumour thickness (measured histologically) were not accepted in this regression analysis (Table 4). Furthermore, a relatively high correlation coefficient (0·73) between maximum elevation and tumour thickness was noticed. The average tumour thickness within the low-risk group, defined by clinical criteria combined (Table 3), was 0·75 mm (range, 0·1–1·5 mm). The average tumour thickness of high-risk cases was 4·2 mm (range, 1·0–21·0 mm). The comparison of clinical and histological prognostication is shown in Fig. 4.

DISCUSSION

This analysis revealed maximum elevation (in mm) to be the most important clinical prognostic indicator in malignant melanoma. The prognostic relevance of this parameter was also shown by Drepper, Lindemann & Obst (1980) and Heite (1981). A close association with tumour thickness (Breslow, 1975) was demonstrated by a relatively high correlation coefficient. The number of cases with minimal elevation and deep invasive growth was very small in this group of patients analysed; only five out of seventy-one patients with maximum elevation less than 1 mm had a tumour thickness greater than 1·5 mm. All of them were excluded from the low-risk group by revealing other adverse clinical criteria. The exact maximum elevation of the tumour above skin surface level is best determined by skin replicas. This method was not performed in this study, and for practical purposes, direct measurement with a millimetre scale appears to be sufficient in most cases.

The use of a combination of several clinical criteria certainly allows a better prognosis to be made; however, a compromise between effectiveness and simplicity in application must be reached. In our analysis surface defects (erosion, ulceration, bleeding) and location allowed additional prognostic information to be gained. Furthermore, the diameter of the lesion was relevant, even though maximum elevation clearly proved to be the dominant indicator; this was

TABLE 5. Additional clinical criteria with a possible prognostic relevance

Favourable	Unfavourable
Low risk with head/neck location	Satellite, particularly when > 1 cm away from the primary
Plateau-like lesion	More than one individual nodule
	Ulceration ⩾80% of the lesion (Day *et al.*, 1982)

also shown by Drepper *et al.* (1980). The diameter of the nodule appeared to be more important than the diameter of the total lesion (also shown by Day *et al.*, 1982).

Other factors with prognostic relevance, but which did not increase significantly the effectiveness of the prognostic classification (Table 3), are given in Table 5. Their limited evaluation was mainly due to too small a number of cases in these particular subgroups. Another limitation of this study was the relatively small portion of low-risk cases. The patients were seen from 1962 to 1972, and the percentage of low-risk cases has increased in our institution during the past decade.

This prognostic classification by clinical criteria has been shown to be as reliable as the use of histological criteria, such as tumour thickness.

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Distribution of p230, an α-spectrin-related polypeptide in normal and psoriatic epidermis and in cultured human keratinocytes

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SUMMARY

We studied the localization of p230, an immunoanalogue of erythroid α-spectrin, in normal and psoriatic human epidermis and in cultured human keratinocytes. In immunofluorescence microscopy of frozen sections of normal human skin a bright cytoplasmic staining was seen in the cortical area of the keratinocytes. Similar staining was also seen in lesional and uninvolved areas of psoriatic epidermis. The pericytoplasmic localization of p230 could also be seen in cultured human keratinocytes: a lamina-like reticular staining was seen mostly confined to the ventral cytoplasmic aspect and to junctional areas of the cells. Immunoblotting of electrophoretically separated polypeptides of epidermal cells revealed a distinct polypeptide of M_r 230 kD. The results indicate that α-spectrin-like polypeptides form a major cytoskeletal framework in human epidermal cells in both normal and psoriatic skin.

Earlier studies indicated that spectrins, the major cell surface-associated cytoskeletal proteins of erythrocytes, would be absent from other cell types (Hiller & Weber, 1977). However, recent results have shown that many cells contain polypeptides closely related to erythroid α-spectrin (Goodman, Zagon & Kulikowski, 1981; Repasky, Granger & Lazarides, 1982; Glenney, Glenney & Weber, 1982; Lehto & Virtanen, 1983), indicating that spectrin-like polypeptides play a general role in the maintenance of cell surface architecture. In addition, non-erythroid spectrin-like molecules appear to be major calmodulin-binding proteins in diverse cell types (Glenney *et al.*, 1982; Carlin, Bartelt & Siekewitz, 1983).

Much interest has been devoted to the presence and functions of calmodulin, the major intracellular calcium receptor (Means & Dedman, 1980) in normal and diseased epidermis (Saurat *et al.*, 1981; Iizuka *et al.*, 1982; Van de Kerkhof & Van Erp, 1983; Pavlovitch *et al.*,

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1983). Recently, elevated levels of calmodulin have been found in lesional skin of psoriatic patients (Van de Kerkhof & Van Erp, 1983).

Using immunofluorescence and immunochemical methods we now show the presence and pericytoplasmic localization of an α-spectrin-like polypeptide, p230, in human epidermal cells. The distribution of p230 appeared to be similar in normal and psoriatic skin.

METHODS

The skin biopsies were taken at surgical operations from different areas of skin of six patients with no history of psoriasis. The biopsies from ten psoriatic patients were taken from untreated psoriatic plaques and uninvolved skin of the arm. Frozen sections were fixed in methanol, cooled to −20°C, for 10 min. Human keratinocyte cultures were prepared from the suction blister roof epidermis as described earlier (Kariniemi *et al.*, 1982). Briefly, enzymatically isolated keratinocytes were cultured on collagen-coated coverslips in MCDB-151 medium supplemented with 10% pooled human AB serum (Finnish Red Cross Blood Service, Helsinki, Finland), hydrocortisone and antibiotics. For immunostaining, the cells were fixed either with 3·5% paraformaldehyde in 0·1 M phosphate buffer, pH 7·4, for 10 min, or in methanol, cooled to −20°C, for 10 min.

For indirect immunofluorescence microscopy, rabbit antibodies against a 230,000 dalton polypeptide (p230), isolated from calf lens, were used. The purification of the p230, antibody production and affinity-purification have been described recently (Lehto & Virtanen, 1983). We have also shown that the affinity-purified p230 antibodies react specifically with the α-subunit of erythroid spectrin (Lehto & Virtanen, 1983). For fluorescence staining, the sections and

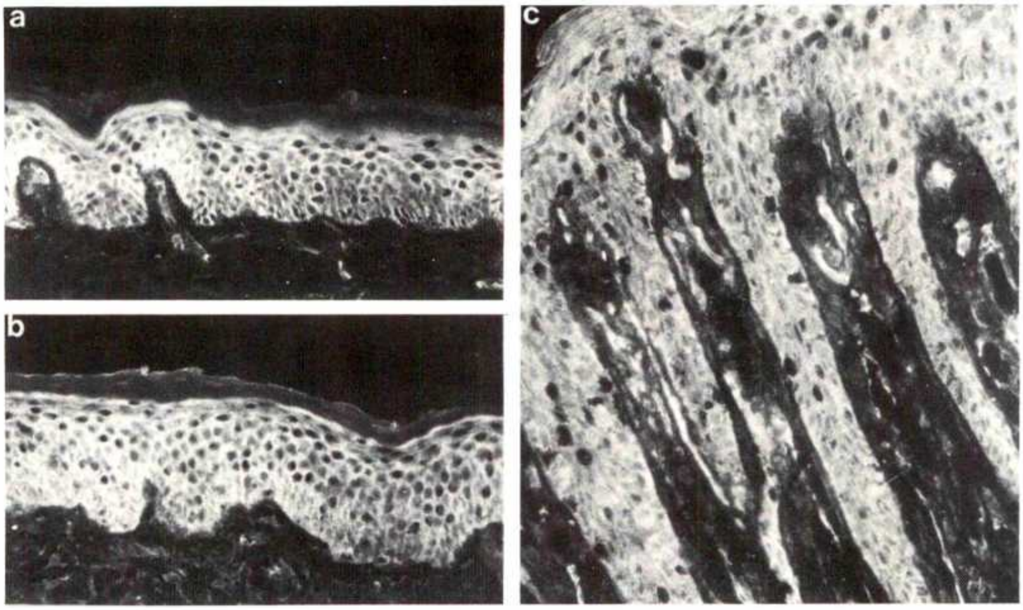

FIGURE 1. Indirect immunofluorescence microscopy (IIF) staining of frozen sections of normal human epidermis (a), of uninvolved psoriatic epidermis (b) and of psoriatic lesion (c) with antibodies to p230. Low magnification shows the presence of p230 in all living epidermal cell layers. (× 120.)

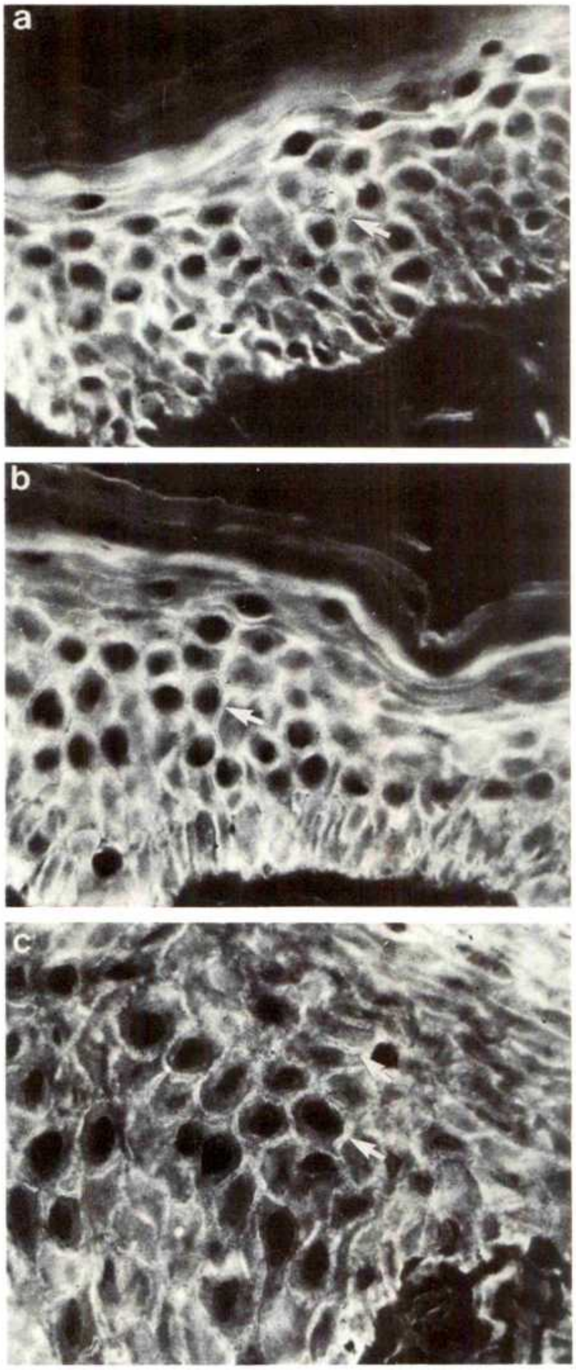

FIGURE 2. At a higher magnification, IIF of frozen sections of both normal (a), uninvolved (b) and involved psoriatic (c) epidermis reveals p230 in a peripheral cytoplasmic localization in keratinocytes. Note the distinct bilaminar localization found in many cells (arrows). (× 340.)

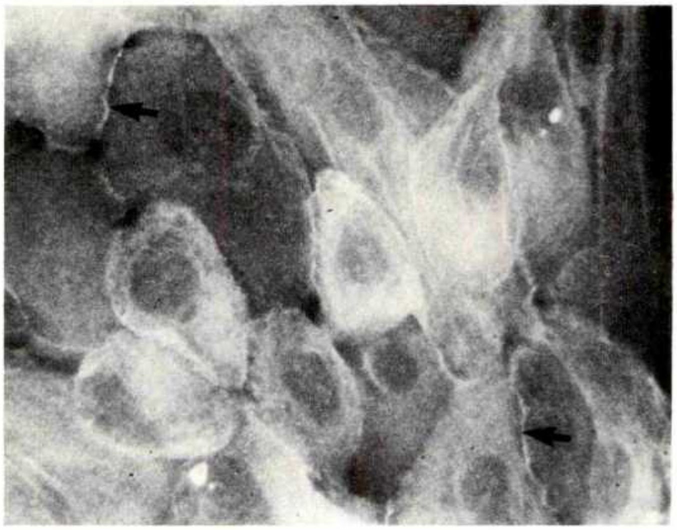

FIGURE 3. In cultured human keratinocytes p230 is seen as a sheet-like reticular localization mostly confined to the ventral aspect of cells but also seen at the cell–cell junctional areas (arrows). (× 400.)

cultured cells were first exposed to the rabbit antiserum, washed and then exposed to fluorescein isothiocyanate-coupled (FITC) goat anti-rabbit IgG antiserum (Cappel Laboratories, Cochranville, Pa.). After washing, the specimens were embedded in Veronal–glycerol buffer, pH 8·4, and examined in a Zeiss Universal microscope equipped with an epi-illuminator III RS and filters for FITC fluorescence.

For the immunoblotting method, pure normal human epidermis was separated from the dermis by digestion with 0·25% trypsin and 0·02% EDTA in phosphate-buffered saline (PBS) at 37°C for 2–2·5 h (Kariniemi *et al.*, 1982). Sheets of epidermis were minced with scissors and extracted with 0·5% Triton X-100 in 50 mM Tris–HCl (pH 7·4) and supplemented with 1 mM phenylmethyl-sulphonylfluoride and 1 mM *N*-α-*p*-tosyl-L-lysine chlormethyl ketone for 30 min at 0°C. Cultured keratinocytes derived from both normal persons and from uninvolved skin of patients with psoriasis were similarly extracted in the above detergent solution. All the specimens were collected by low speed centrifugation. After solubilization in the electrophoresis sample buffer, the specimens were run in polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (Laemmli, 1970) using 6·5% slab gels. Thereafter, the polypeptides were transferred on to nitrocellulose paper according to Towbin, Staehelin and Gordon (1979). For protein staining, 0·1% amido black was used (Towbin *et al.*, 1979). For immunostaining, the nitrocellulose sheets were first incubated overnight in 3% albumin–PBS solution and stained with anti-p230 antibodies as described earlier (Lehto & Virtanen, 1983). Molecular weight standards (ferritin, 220,000 daltons; phosphorylase b, 94,000 daltons; albumin 68,000 daltons; ovalbumin, 43,000 daltons) were from Pharmacia Fine Chemicals (Uppsala, Sweden).

RESULTS

In immunofluorescence microscopy of frozen sections of normal human skin, the p230 spectrin immunoanalogue was found in all epidermal cell layers except the keratinized layer (Fig. 1a).

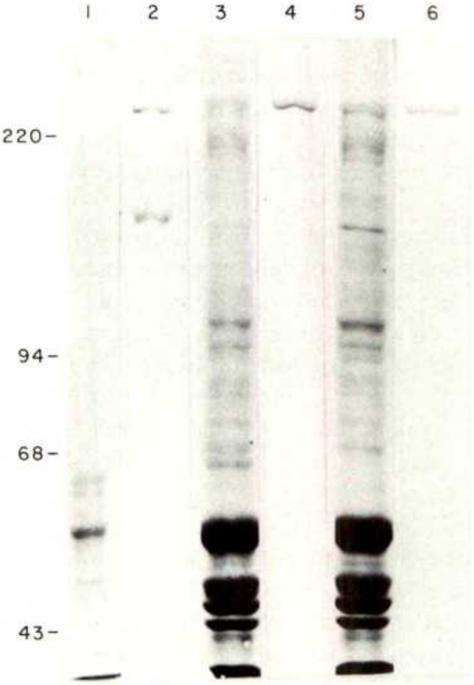

FIGURE 4. Immunoblotting of epidermal and keratinocyte polypeptides with antibodies to p230. Lane 1, epidermal polypeptides on nitrocellulose sheets after staining with amido black. Lane 2, immunostaining of epidermal polypeptides with p230 antibodies reveals a prominent polypeptide with Mr 230 kD. Lanes 3, 5, polypeptide of cultured normal human keratinocytes and keratinocytes from uninvolved psoriatic epidermis, respectively. Immunostaining with p230 antibodies reveals a prominent p230 polypeptide in both cells (lanes 4, 6). Note the presence of a prominent immunoreactive 140 kD polypeptide among epidermal polypeptides (lane 2) which is much fainter in cultured keratinocyes. Molecular weight standards (10^3 daltons) are indicated on the left.

Similarly, an even immunostaining of epidermal cell layers was also seen in frozen sections of uninvolved psoriatic skin (Fig. 1b) as well as of psoriatic lesions (Fig. 1c). Interestingly, in psoriatic lesions the staining appeared less intense than in normal skin. Higher magnification showed that the p230-specific staining was confined to cell periphery often giving a bilaminar staining of cell peripheries in normal skin (Fig. 2a), in uninvolved psoriatic skin (Fig. 2b) and in psoriatic lesions (Fig. 2c).

The localization of p230 was also studied in cultured human keratinocytes. No immunostaining was seen in cells fixed in paraformaldehyde (not shown, see Lehto & Virtanen, 1983), indicating that the antigen is not exposed at the cell surface, whereas cells subsequently made permeable with 0·1% Nonidet P40 or fixed in cold methanol, showed a bright lamina-like staining, confined mostly to the ventral cytoplasmic aspect of the cells, but found also at junctional areas of cells (Fig. 3).

In immunoblotting of electrophoretically separated polypeptides of normal human epidermis and of cultured keratinocytes from normal and uninvolved psoriatic skin, p230 antibodies gave a distinct reaction with a polypeptide of M_r 230,000 daltons (Fig. 4, lanes 1–6). In addition, immunostaining revealed a polypeptide of M_r 140,000 daltons in all specimens, apparently representing a degradation product of α-spectrin (Carlin *et al.*, 1983).

DISCUSSION

There is much interest in the role of cytokeratin-type intermediate filaments, microtubules and microfilaments in the organization and function of keratinocytes (Sun & Green, 1978; Kariniemi *et al.*, 1982; Kunzenbacher *et al.*, 1982; Jones *et al.*, 1982). Little is known about the organization of cell surface proteins and their interactions with cytoskeletal elements in epidermal cells.

Recently, interactions of spectrins and spectrin-like proteins with cell membranes have been clarified in different cells. In erythrocytes, spectrins are known to form an intricate network at the cytoplasmic aspect of the cell surface membrane, interacting with actin and some other cytoskeletal proteins. Also in non-erythroid cells, α-spectrin-like molecules appear to form a lamina-like framework under the plasma membrane (Goodman *et al.*, 1981; Repasky *et al.*, 1982; Glenney *et al.*, 1982; Lehto & Virtanen, 1983). Our results show that α-spectrin-like polypeptides are also the major cell surface-associated cytoskeletal proteins in human keratinocytes.

Spectrins and spectrin-like proteins appear to be the major calmodulin-binding proteins in several different cell types (Glenney *et al.*, 1982; Carlin *et al.*, 1983), thus apparently participating in many calcium-mediated cell functions (see Kakiuchi & Sobue, 1983). Earlier studies have shown that calmodulin, the major intracellular calcium receptor (Means & Dedman, 1980), is present in mammalian keratinocytes (Iizuka *et al.*, 1982), and elevated levels of calmodulin have been reported in diseased psoriatic epidermis (Van de Kerkhof & Van Erp, 1983). In the present study, however, no difference could be seen either in the distribution or intensity of the α-spectrin-specific immunostaining between normal and uninvolved psoriatic epidermis. In psoriatic lesions, however, the staining was not so intense as in normal skin, which was unexpected in view of the findings on elevated calmodulin levels in such lesions (Van de Kerkhof & Van Erp, 1983).

Recent studies by Saurat *et al.* (1981) and Pavlovitch *et al.* (1983) suggested that the major calcium-binding protein of skin is confined solely to the basal epidermal cells and would be present only in some other proliferative cells. It remains to be elucidated therefore whether there are two distinct calcium-binding protein systems in skin, one interacting with α-spectrin-related polypeptides and present throughout the whole epidermis, and another solely confined to the basal epidermal cells.

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Neutral proteinases and other neutrophil enzymes in psoriasis, and their relation to disease activity

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SUMMARY

The activities of elastase, cathepsin G, lysozyme and myeloperoxidase of polymorphonuclear leukocytes were determined by spectrophotometry in thirty-six patients with psoriatic lesions, twelve symptom-free patients with psoriasis and fifteen normal controls. The mean activities of cathepsin G, elastase and lysozyme were found to be increased by 55 to 70% in patients with actively spreading plaque lesions compared with healthy controls ($P < 0{\cdot}01$). Most patients with guttate lesions had total enzyme activities within the normal range. Those with stationary plaque psoriasis had activities of both neutral proteinases (cathepsin G and elastase) which were about 40% lower than normal controls ($P < 0{\cdot}05$). In the lesion-free psoriatics, the activities of neutral proteinases were about 70% of control values.

Our findings emphasize the importance of assessment of disease activity in this sort of investigation. The present data may help to resolve much of the confusion regarding PMN function in psoriasis.

Polymorphonuclear leukocytes (PMNs) seem to play an essential role in the pathogenesis of psoriasis. The psoriatic epidermis contains excessive amounts of several chemo-attractants such as hydroxyeicosatetraenoic acid (HETE) (Hammarström *et al.*, 1975), neutral proteinases (Lazarus, Yost & Thomas, 1977), C5a (Tagami & Ofuji, 1977), complement binding stratum corneum (SC) antigen-SC autoantibody complex (Beutner *et al.*, 1981), etc., and it is infiltrated with multiple PMNs which may form microabscesses (Pinkus & Mehregan, 1966).

Numerous data suggest there is dysfunction of PMNs in psoriasis. These cells are hyper-responsive to various chemo-attractants (Wahba *et al.*, 1978; Michaelsson, 1980; Preissner, Schröder & Christophers, 1983) and they show increased adherence (Sedgwick,

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Bergstresser & Hurd, 1980) and enhanced phagocytic activity (Wahba *et al.*, 1978). Furthermore, oxidation in PMNs is stimulated by psoriatic sera (Sedgwick, Bergstresser & Hurd, 1981). Elimination of PMNs in dialysis fluid may be responsible for the clearing of psoriasis by peritoneal dialysis (Gliński *et al.*, 1980).

The activities of neutral serine proteinases of peripheral blood PMNs have been found to be either elevated or normal in active plaque psoriasis (Gliński *et al.*, 1980; Fraki *et al.*, 1983). Besides the property of direct generation of C5a (Lazarus *et al.*, 1977), the PMN neutral proteinases could potentiate the SC autoantibody binding to the horny layer due to conversion of SC antigen to the reactive form (Beutner *et al.*, 1981).

Since the findings on the activities of neutral serine proteinases of PMNs in psoriasis are divergent, the present study was designed to measure the activities of some granule enzymes of PMNs in psoriatic patients with respect to disease activity, duration of recent relapse, and extent of psoriatic lesions.

METHODS

Forty-eight patients with psoriasis vulgaris (thirty-six with lesions and twelve symptom-free) and fifteen age- and sex-matched healthy volunteers were studied. The patients had not received systemic medication for at least 3 months and topical treatment was withdrawn 2 weeks before the test.

The criteria of disease activity were based on the character of the psoriatic lesions, the dynamics of their spreading and to some extent on the time of reappearance of the lesions. The grade of disease activity was defined as follows:

(1) Active, guttate psoriasis (A2)—widely disseminated small papules (not exceeding 5 mm) with some pin-point lesions and occasional small plaques; lesions of duration usually up to 1 month after relapse. (2) Active plaque psoriasis (A1)—predominantly large plaques, intensively growing on the periphery, small papules just perceptible; no pin-point lesions.

(3) Stationary psoriasis (AO)—large plaques for more than 3 months without peripheral spreading, as well as indolent lesions at typical locations.

Chemicals

Micrococcus lysodeicticus (dried cells), peroxidase (horseradish), lysozyme (egg white), and *N-tert*-butoxycarbonyl-L-alanine-*p*-nitrophenyl ester (BANA) were obtained from Sigma; *N*-benzoyl-tyrosine ethyl ester (BTEE) from Calbiochem; *o*-dianisidine from Fluka; Folin Ciocalteu reagent from Merck; Triton X-100 from BDH; Tris from Koch-Light Lab., gelatine from Loba Chemie Fischamend.

Peripheral blood PMNs, separation and elution

The separation of the cells was as described earlier (Gliński *et al.*, 1980). The yield of leukocytes was about 50%; approximately 85% of them were PMNs of more than 95% viability, as determined by trypan blue exclusion. All experimental results were corrected in every patient according to his PMN count in a final suspension, and are presented as the enzymatic activity per 10^6 PMNs.

Elution of PMNs was performed at 4°C using a density of 10^8 cells per ml of buffer. The cells were suspended twice (overnight and for 2 h) in 0·05 M citrate buffer, pH 3·5 (fraction A), and next eluted twice (overnight and for 2 h) with 0·1% Triton X-100 in the same buffer (fraction B). The eluates were analysed separately.

Biochemical measurements

Protein was determined using a microadaptation of the Lowry method with 1% sodium citrate–$CuSO_4$ solution and bovine serum albumin as a standard.

Lysozyme activity was measured as previously (*Worthington Enzyme Manual*, 1972), except that 0·066 M sodium phosphate buffer, pH 6·6, was used to suspend *M. lysodeicticus* cells.

Determination of myeloperoxidase (MPO) was based on the *Worthington Enzyme Manual*, 1972.

The activity of cathepsin G was tested against BTEE at 31°C. The incubation mixture (1·2 ml) contained: 0·1 ml of 1 M Tris–HCl buffer, pH 8·0; 0·05 ml KCl; 0·02 ml of BTEE (3.14 mg/ml in 50% methanol); 0·1 ml of eluate; and water to 1·2 ml. ΔE_{256} was followed and the initial as well as the maximal velocities were evaluated using the values 964 M^{-1} cm^{-1} for the E_{256} (Kang & Fuchs, 1973) and 2·6 mM for the K_m (Starkey & Barrett, 1976).

Elastase activity was tested against BANE at 31°C. The incubation mixture (1·25 ml) contained 1 ml of 0·2 M Tris–HCl buffer, pH 7·5; 0·1 ml of 1 M KCl; 0·1 ml of 0·2 M $CaCl_2$; 0·04 ml of BANE (4·2 mg/ml in methanol); 0·005 to 0·075 ml of eluate; and citrate buffer to 1·25 ml. $\Delta E_{347\,5}$ was corrected for spontaneous BANE hydrolysis. $E_{347\,5}$ of $5{\cdot}5 \times 10^3$ M^{-1} cm^{-1} (Visser & Blout, 1972) and a K_m of 0·32 mM were used in calculations. The K_m (0·32 ± 0·01 mM) was determined on the PMN eluates from three healthy subjects and calculated according to Wilkinson (1962).

RESULTS

PMN granule enzymes in psoriatics and normals

Increased soluble proteins were found in patients with psoriasis, but there were no significant differences in the enzymatic activities between fifteen normal controls and thirty-six psoriatics

TABLE 1. Mean values of PMN granule enzyme activities in healthy subjects and psoriatics related to the presence of skin lesions and disease activity

Group	No. of cases	Protein mean ± s.d.	Enzyme activity			
			Lysozyme mean ± s.d.	MPO mean ± s.d.	Elastase mean ± s.d.	Cathepsin G mean ± s.d.
Total patients with psoriatic lesions	36	72·3 ± 33·6	44·0 ± 31·2	0·096 ± 0·083	1·48 ± 0·87	2·85 ± 1·59
A2	12	60·5 ± 9·1	30·4 ± 18·8	0·089 ± 0·049	1·14 ± 0·48	2·58 ± 1·18
A1	14	96·7 ± 34·2^{a}	60·4 ± 27·5^{a}	0·103 ± 0·113	2·18 ± 0·91^{a}	4·04 ± 1·55^{a}
A0	10	47·6 ± 25·3	33·9 ± 37·4	0·091 ± 0·070	0·88 ± 0·47^{b,c}	1·51 ± 0·63^{b,c}
Patients without skin lesions	12	57·8 ± 21·2	38·3 ± 17·3	0·098 ± 0·085	0·93 ± 0·44^{b,c}	1·75 ± 0·96^{c}
Normal controls	15	53·5 ± 15·7	38·8 ± 17·6	0·135 ± 0·129	1·34 ± 0·45	2·37 ± 0·98

All data are normalized for 10^6 PMNs. Elastase and cathepsin G activities in nanokatals, lysozyme and MPO in units; and protein in micrograms.

Statistical significance of the difference from normal controls (*t*-test):

[a] Increase, $P < 0{\cdot}01$;

[b] Decrease, $P < 0{\cdot}05$.

Difference from total psoriatics with skin lesions:

[c] Decrease, $P < 0{\cdot}05$.

with skin lesions (Table 1). In the symptom-free psoriatics, the activities of elastase and cathepsin G were decreased compared with both patients with psoriatic lesions and healthy subjects.

Activity of PMN granule enzymes related to the activity of the disease

In active guttate psoriasis (A2), there was a slight increase of cathepsin G activity compared with normals. In contrast, there was a tendency towards a reduction in the mean activities of elastase and lysozyme in comparison with healthy subjects (Table 1).

When plaque lesions were active and peripherally spreading (A1) the mean activities of elastase, cathepsin G, and lysozyme were significantly elevated (about 55–70% above the control values); this is similar to the protein content in the eluates. The activity of MPO in these patients still remained lower than in normal volunteers.

A marked decrease in the activities of both proteinases, elastase and cathepsin G, was demonstrated in patients with stationary psoriatic lesions (AO) in comparison with healthy subjects.

Activity of PMN granule enzymes related to the duration of recent relapse of psoriatic lesions

A tendency was observed that the longer the duration of relapse, the higher were the activities of PMN lysozyme, elastase and cathepsin G; however, these differences did not reach statistical significance. The activities of elastase (2·47 ± 0·91 nkat) and cathepsin G (4·54 ± 1·59 nkat) were significantly increased ($P < 0{\cdot}002$) only in patients with active plaque lesions (A1), which lasted more than 4 months. In contrast, despite duration of the lesions above 4 months, both these proteinases (0·70 ± 0·33 nkat and 1·37 ± 0·64 nkat respectively) were significantly ($P < 0{\cdot}05$) less active in patients with stationary plaques compared with normals.

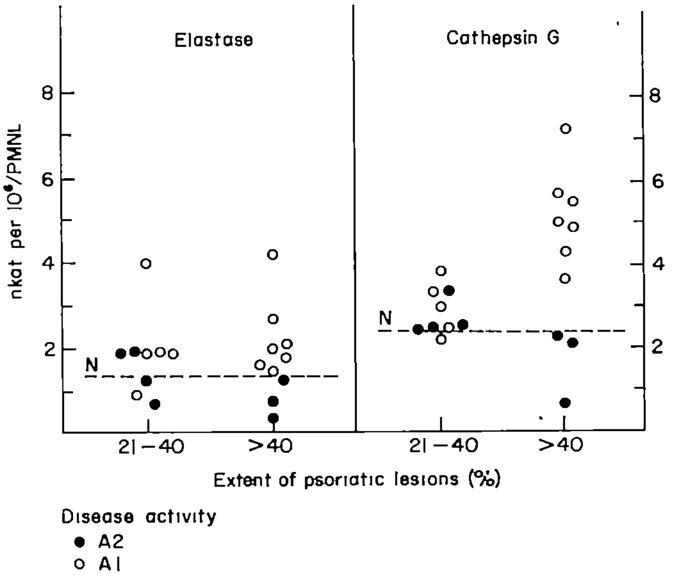

FIGURE 1. The activities of neutrophil elastase and cathepsin G in patients with active guttate (A2) and active plaque (A1) psoriasis in relation to the extent of the lesions.

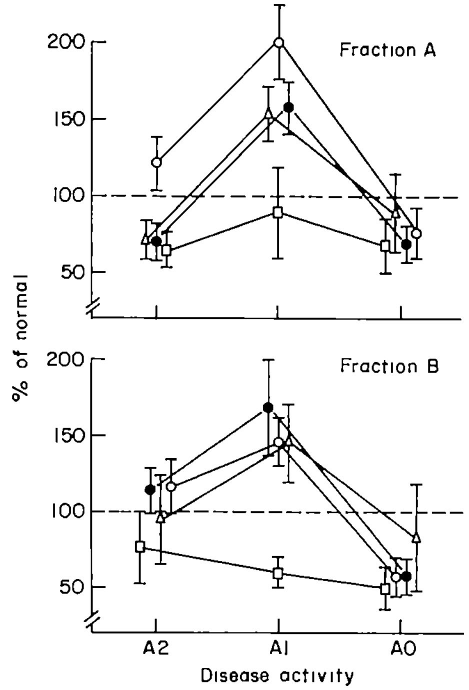

FIGURE 2. Elution of granule enzymes from PMNs at pH 3·5 by citrate buffer alone (Fraction A) and buffered Triton X-100 (Fraction B) in patients with various activity of psoriatic lesions compared with normal controls. (●) Elastase, (○) cathepsin G, (△) lysozyme, (□) MPO. Enzyme activities (mean ± s.e.m.) significantly different from controls (t-test) are as follows. A2, Fraction A: elastase $P < 0·05$, lysozyme $P < 0·05$. A1, Fraction A. elastase $P < 0·005$, cathepsin G $P < 0·002$, lysozyme $P < 0·02$; Fraction B: elastase $P < 0·05$, cathepsin G $P < 0·05$. A0, Fraction A: elastase $P < 0·05$; Fraction B: elastase $P < 0·02$, cathepsin G $P < 0·05$.

Activity of PMN granule enzymes as related to the extent of psoriatic lesions

The mean activities of both neutral proteinases, elastase (1·81 ± 0·61 nkat) and cathepsin G (4·14 ± 1·95 nkat) were significantly elevated ($P < 0·05$ and $P < 0·02$, respectively) in patients with more than 40% of skin surface involved due to the classification of patients with active plaque psoriasis (A1) to this group (Fig. 1). Other correlations between the enzymatic activities and the percentage of skin involved have not been determined.

Recovery of different fractions of PMN granule enzymes in patients with various disease activity

In patients with active guttate psoriasis (A2), the activity of cathepsin G in citrate buffer fraction A, as well as in citrate buffer–Triton fraction B, were slightly increased in comparison with normals (Fig. 2). The activities of elastase and lysozyme were significantly lower ($P < 0·05$) in these patients than in normal controls, but only in fraction A.

Elastase, cathepsin G, and lysozyme were elevated to the same level in both A and B fractions of enzymes in patients with active peripherally spreading plaques (A1). In contrast, A and B fractions of elastase and cathepsin G were significantly reduced only in patients with stationary psoriatic lesions (A0).

In remission, the symptom-free patients had reduced activities of elastase and cathepsin G to the same extent (about 70% of control) in both fraction A and fraction B of the enzymes (data not shown).

DISCUSSION

The separation of granule enzymes by repeated washing of PMNs with 0·05 M citrate buffer minimized (in contrast to homogenized material) the contamination of the eluates with cytoskeleton fragments and other proteins as well as enzymes not solubilized in this medium. These experimental conditions (low pH and low ionic strength) were similar to those proposed by other authors for the solubilization of some neutral proteinases of PMNs (Ohlsson, 1978; Olsson *et al.*, 1978; Rindler-Ludwig, Bretz & Baggiolini, 1978). Sequential elutions in our modification differentiated between readily released (fraction A) and membrane-bound (fraction B) pools of granule enzyme.

The mean total enzymatic activities of the psoriatic group as a whole did not differ from those of the controls because of the wide ranges. This spread was particularly evident in the psoriatic population, and this is in agreement with other studies (Rausch & Moore, 1975; Krueger, Hill & Jederberg, 1978). Significant differences in the activities of granule enzymes were evident, however, when enzyme subfractions in some subgroups of the patients were analysed. Patients with active guttate psoriasis (A2) showed reduced activities of elastase and lysozyme, which concerned predominantly the readily released (unstable) pool of these enzymes. This could result from partial extracellular degranulation of the PMNs (Schmidt, 1978) by some unknown factor(s) operating *in vivo* at the time of spreading of guttate lesions. This possibility is supported by the analogous distribution of these PMN enzymes in the cells degranulated during leukopheresis (data not shown). Alternatively, the initial resting pool of these granule enzymes could be lower in active guttate psoriasis than in normal controls, similar to the psoriatics in remission.

If present, *in vivo* degranulation of PMNs in guttate psoriasis might be a primary response to this factor(s), and in consequence could induce an overproduction of proteolytic and other PMN granule enzymes; the total activities of these enzymes were doubled in active plaque psoriasis (A1), which usually follows guttate psoriasis. Excessive storage and/or deficiency of specific inhibitors is also possible.

The active plaque form of psoriasis has been characterized also by a high amount of acid-soluble proteins (suggesting an excessive production not only of neutral proteinases, but also other proteins of possible pathogenic importance) as well as by a two-fold increase in the proteolytic activity of PMN enzymes (Gliński *et al.*, 1980) and enhanced *in vivo* mobilization of PMNs (Tigalonowa, Gliński & Jabłonska, 1983).

The increased activity of neutral proteinases of PMNs penetrating the epidermis in these cases might be responsible for greater epidermal inflammation inducing chemotaxis (Lazarus *et al.*, 1977) and/or non-specific qualitative changes in the SC (Beutner *et al.*, 1981). Activation of the PMN enzymes may thus play an essential role in the maintenance and persistence of the plaque lesions.

Increased activities of elastase, cathepsin G and lysozyme in both enzymatic fractions from active plaque psoriasis were replaced by about 40% reductions of neutral proteinase activities in stationary plaque psoriasis (A0). Similarly, a reduced *in vivo* mobilization of PMNs has been found to accompany stationary lesions (Tigalonowa *et al.*, 1983). It seems likely that a fall in the neutral proteinase activities could lead to a remission of psoriasis or to a transition of active spreading lesions to stationary plaques.

A discrepancy between our data and the normal values of neutral proteinases of PMNs reported by Fraki *et al.* (1983) could be related to patient selection. About half of the patients studied by these authors had psoriatic arthritis and belonged possibly to the stationary psoriasis group.

Our data argue against an altered function of PMNs as the cause of psoriasis (Wahba *et al.*, 1978; Michaelsson, 1980; Sedgwick *et al.*, 1980; Preissner *et al.*, 1983; Tigalonowa *et al.*, 1983), which seems to be related to disease activity. The relation of neutral proteinase activities to the duration and extent of psoriatic lesions is clearly a secondary phenomenon. After healing of psoriatic lesions the activities of elastase and cathepsin G became normal or even about 30% lower than in healthy subjects.

It may be concluded that (a) an *in vivo* stimulation of PMN granule enzymes and/or dysfunction of regulatory mechanisms, rather than an excessive genetic response of PMNs is involved in active widespread plaque psoriasis, and (b) the increased inhibition of the system of neutral proteinases may be characteristic of the stationary form of the disease.

The mechanism responsible for the stimulation and depression of various granule enzymes of PMNs, including cathepsin G and elastase, in some phases of psoriasis remains to be elucidated with special regard to genetic factors and to the enzyme regulatory system of PMNs.

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Does UV-A potentiate 'short contact' dithranol therapy?

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SUMMARY

The effect of ultraviolet-A (UV-A) irradiation in combination with 'short contact' dithranol treatment was studied in ten patients with chronic plaque psoriasis. Increasing concentrations of dithranol were applied daily to the psoriatic plaques on both sides of the body. One side was irradiated with UV-A daily. There was no difference in the rate of clearance between the two sides. The effectiveness of 'short contact' dithranol was confirmed.

Topical dithranol has maintained a key place in the treatment of psoriasis for many years and is an essential element of the Ingram regime (Ingram, 1953). The conventional formulation in zinc and salicylic acid paste BP (Lassar's Paste) is highly effective but is unacceptable for regular use by out-patients.

Recently a new regime has been advocated using applications of high concentrations of dithranol with salicylic acid in yellow soft paraffin for short periods daily (Runne & Kunze, 1982). They found a more rapid clearance time than with conventional dithranol therapy. This regime stems from the finding that maximum penetration into the epidermis of topically applied dithranol occurs within the first hour (Selim *et al.*, 1981). Furthermore, penetration of dithranol into psoriatic lesions is more rapid than into normal skin (Schalla, Bauer & Schaffer, 1981).

Oral psoralen and long-wave ultraviolet irradiation (PUVA) is an effective form of therapy for psoriasis (Parrish *et al.*, 1974; Wolff *et al.*, 1976), but reports of cutaneous carcinomata in patients treated with PUVA (Stern *et al.*, 1979; Reshad *et al.*, 1984) raise the question of its long-term safety. The combination of PUVA with topical dithranol treatment has been claimed to reduce the cumulative PUVA exposure (Cripps & Lowe, 1979).

Following a study of the absorption spectra of UVA-irradiated and non-irradiated dithranol, Hindson *et al.* (1983) have described the use of short-contact dithranol therapy, in combination with UV-A (DUVA). A favourable clearance time was reported, but the study did not include non-irradiated controls treated with short-contact dithranol therapy alone. We have therefore conducted a half-body comparison study to assess any potentiation of short-contact dithranol therapy by UV-A irradiation.

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TABLE 1. Treatment protocol

Days 1 and 2	0·25% Dithranol + 2% salicylic acid in yellow soft paraffin
Days 3 and 4	0·5% Dithranol + 2% salicylic acid in yellow soft paraffin
Days 5 and 6	1% Dithranol + 2% salicylic acid in yellow soft paraffin
Days 7,8 and 9	2% Dithranol + 2% salicylic acid in yellow soft paraffin
Day 10 onwards	3% Dithranol + 2% salicylic acid in yellow soft paraffin

METHODS

Ten adult patients with chronic plaque psoriasis of sufficient severity to merit hospital admission were studied. Patients with a symmetrical pattern of psoriasis were chosen to allow a half-body comparison. Patients receiving potentially photosensitizing drugs or with a history of photosensitivity were excluded. Informed consent was obtained. A combination of dithranol with 2% salicylic acid in yellow soft paraffin was applied to the lesions daily and was left on the skin for one hour. This was then washed off in a bath using Teepol® detergent (Shell) and Oilatum Emollient® (Stiefel). The concentrations of dithranol used are shown in Table 1.

Within 1 hour of removal of the dithranol the left side was exposed to UV-A at a dose of 10 J/cm^2 daily (Monday–Friday) whilst the right side of the body was covered by material which was shown to be impenetrable to UV-A. UV-A treatment was administered in a cylindrical cabinet (London Photochemotherapy apparatus) containing 40 Philips TL 95/100W 09N lamps, 175 cm in length. No coal tar solution was used at any stage but scalp psoriasis was treated with a coal tar and salicylic acid ointment (Leeds pomade®, McCarthy's) and flexural lesions with 0·05% clobetasone butyrate ointment (Eumovate®, Glaxo).

Treatment was continued daily until the psoriasis cleared. In patients who failed to make satisfactory progress, treatment was changed to the Ingram regime. Clinical assessment was performed twice weekly and the time taken to clear the psoriasis was noted.

TABLE 2.

			Clearance (days)	
Patient	Sex	Age (years)	Left side (UVA-irradiated)	Right side (Not irradiated)
1	F	20	15	15
2	F	16	12	12
3	F	60	Withdrawn	Withdrawn
4	M	46	14	14
5	M	63	11	11
6	M	47	16	16
7	F	34	7	7
8	M	15	19	19
9	F	30	19	19
10	F	64	Withdrawn	Withdrawn
		Median 40	Median 14·5	

RESULTS

Details of the patients are presented in Table 2. Eight out of ten patients attained complete or almost complete clearance during the study period in a median time of 14·5 days (range 7–19 days). There was no difference in the rate of clearance between the two sides and slight pigmentation of the irradiated side was the only detectable difference at the completion of the study. The 95% confidence interval for eight readings was between 0 and 37%.

Two patients failed to make satisfactory progress. Their treatment was changed to the Ingram regime after 15 and 21 days.

DISCUSSION

Our results support the claim by Runne & Kunze (1982) that daily short-contact therapy with dithranol is an effective treatment for psoriasis. The median clearance time of 14·5 days compares with 15 days for the standard Ingram regime used in this hospital (unpublished data) and is faster than the mean of 33 days found with the 3-hour dithranol therapy described by Runne and Kunze (1982). However, in the present study a higher concentration of dithranol was used initially and the concentration was increased more quickly.

We found no difference in the rate of clearance between the side exposed to UV-A and the unexposed side in eight patients. Although the number of patients treated was small it is clear that the majority of patients are unlikely to benefit from this regime and we cannot confirm the claims of Hindson *et al.* (1983). It is possible that a higher dose of UV-A than that used in this study would have had a noticeable effect. However, the clearance times we obtained were very satisfactory and it would be difficult to justify the theoretical risk of higher UV-A exposure.

Our clearance rate is very similar to that obtained by Hindson *et al.* (1983) who reported a median clearance time of 14 days (range 7–39 days). In both studies dithranol was applied in yellow soft paraffin with 2% salicylic acid and left in contact with the skin for 1 hour, although there was a slight difference in the starting concentration and the rate of increase. Both studies show appreciably shorter clearing times than either of the short-contact dithranol regimes described by Runne and Kunze (1982). The 3-hour regime showed a mean clearance time of 33 days in fifteen patients and the 'minutes' therapy (maximum 20 minutes) a mean clearance time of 26·9 days.

Our study confirms the efficacy of short-contact dithranol therapy but has shown no benefit from additional UV-A exposure. There is clearly a need for further study of the concentration of dithranol and duration of contact required to produce optimum clearance rates in psoriasis.

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Effects of *in vitro* PUVA on human leukocyte function

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SUMMARY

Human polymorphonuclear and monomorphonuclear leukocytes (PMNL and MMNL) were exposed *in vitro* to 8-methoxypsoralen (8-MOP, 0·1–80 μg/ml) and/or UV-A radiation (0·03–2 J/cm^2) and then analysed for the following functions: chemotaxis, bactericidal activity and proliferation in response to mitogen stimulation. The functions of PMNL became depressed only at a high PUVA dose level (about 20 μg/ml of 8-MOP plus 2 J/cm^2 of UV-A), whereas with MMNL chemotaxis was inhibited at 1 μg/ml of 8-MOP plus 2 J/cm^2 of UV-A and lymphocyte proliferation was diminished at 0·1 μg/ml plus 0·1 J/cm^2.

Since with the MMNL, as compared with the PMNL, a longer time period was present between PUVA exposure and analysis, and since no difference between these cell types in trypan blue exclusion could be seen, the relative sensitivity of the MMNL functions was taken as evidence of DNA damage being a mechanism for the observed PUVA-induced effects.

PUVA chemotherapy, i.e. oral psoralens and long-wave ultraviolet radiation (UV-A), is widely used in the treatment of several dermatoses including psoriasis and mycosis fungoides (Parrish, 1981).

It has been estimated that during PUVA therapy the peripheral leukocytes, while circulating through the dermis, will be exposed to approximately 1–5% of the skin surface UV-A dose (i.e. about 0·1 J/cm^2) in combination with the concentration of 8-MOP attained in blood (about 0·1 μg/ml) (Kraemer, 1982). Evidence that dermal lymphoid cells may be affected by PUVA *in vivo* is provided by the beneficial effect of PUVA in mycosis fungoides (Parrish, 1981). Also the ability of PUVA to clear psoriasis may suggest an influence of PUVA on dermal leukocytes since immunological abnormalities appear to have some role in the pathogenesis of psoriasis (Jabłońska, Gliński & Beutner, 1982). Further evidence of the effects of PUVA on the immune system has been provided by the findings of diminished responses to dinitrochlorobenzene and depressed lymphocyte proliferation in PUVA-treated psoriasis patients (Moss, Friedmann &

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Shuster, 1981; Kraemer, 1982). Although studies on the influence of PUVA on PMNL functions are infrequent, chemotaxis has been reported as being impaired by *in vitro* treatment (Langner & Christophers, 1977), whereas no such effect with *in vivo* therapy has been noted (Silny *et al.*, 1980). Part of the influence of PUVA therapy on human leukocyte function may be due to the UV-A component *per se*, since solarium exposure has been shown to increase the ratio of suppressor/helper subsets of T lymphocytes (Hersey *et al.*, 1983).

In the present study PMNL and MMNL were exposed *in vitro* to wide dose ranges of 8-MOP, UV-A and PUVA in order to determine which leukocyte functions are affected during clinical photochemotherapy.

METHODS

Cells. Heparinized blood from healthy laboratory personnel was used in all experiments. Suspensions of MMNL were prepared by diluting the blood with one volume of RPMI 1640 (Gibco Bio-Cult Ltd, Edinburgh, Scotland), centrifugation on Ficoll–Isopaque (Lymphoprep, Nyegaard & Co A/S, Oslo, Norway) at 1600 r.p.m. for 15 min, harvesting interphase cells, washing twice in RPMI 1640 and finally suspending the cells in serum-free RPMI enriched with 0·2% albumin, 2 mmol/ml of L-glutamine, and 12 μg/ml of gentamicin (culture medium). Suspensions of PMNL were prepared by dextran (2% Dextran 70) (Pharmacia, Uppsala, Sweden) sedimentation of the blood. Erythrocytes were removed by osmotic shock, as described by Davies, Whittaker & Khurshid (1976). The PMNL were then washed three times in phosphate-buffered saline, pH 7·2, and finally suspended in buffer or culture medium.

Treatment with 8-MOP and/or UV-A. Stock solutions of 4 mg 8-MOP (Sigma Chemical Co., St Louis, Missouri, U.S.A.) per ml in 95% ethanol were used. Final concentrations of ethanol did not exceed 0·5%. No influence on chemotaxis, phagocytosis or lymphocyte proliferation due to this solvent was noted. Cells were incubated in the presence of 8-MOP for at least 30 min in darkness at 37°C before UV-A exposure. Two Philips TL 08/40W UV-A bulbs were mounted 10 cm from the cells. The radiation was filtered through 3 mm thick window glass to give an intensity of 1·8 mW/cm^2 at the cell level (Black-Ray PUVA-meter, Waldmann Werk Für Lichttechnik, Schwenningen, W. Germany). The energy can be estimated to peak at about 350 nm, to reach into the visible field and to contain no more than 0·1% of energy as wavelengths less than 320 nm; the spectrum of another specimen of this type of UV-A source has been determined (Bredberg, 1981). The walls of the plastic tubes containing the cells in some of the experiments transmitted about 70% of the energy. After PUVA treatment exposure of the cells to ambient light was kept to a minimum.

In the experiment shown in Table 2, the cells were subjected to an extensive washing procedure consisting of centrifugation (1200 r.p.m., 10 min), suspension in Hank's balanced salt solution, centrifugation, suspension in culture medium, incubation for 30 min to 1 h at 37°C, centrifugation and finally suspension in culture medium.

Chemotaxis. Leukocyte chemotaxis was studied by the method of Nelson, Quie & Simmons (1975) as modified by Forsgren and Schmeling (1977). Agarose plates in 60 mm tissue culture dishes were prepared as described previously (Hallengren & Forsgren, 1978). In each dish six series of wells with a diameter of 3 mm were cut. One-half ml of a suspension of about 10^8 PMNL or MMNL per ml was treated with 8-MOP and/or UV-A while kept in plastic tubes. Cells (10 μl) were then added to the inner wells of the agarose plates, while the six outer wells were all filled with 10 μl of zymosan-activated serum (MMNL) or supernatant from *E. coli* suspensions (PMNL). Incubation of the PMNL was carried out for a period of 3 h at 37°C in a humidified atmosphere containing 5% CO_2 in air. No migration of MMNL could be seen after 3 h and the distance of migration was therefore determined at a later time—after 24 h. The plates were then fixed and stained. Chemotaxis was quantitated by measurement of a greatly enlarged projection of the migration patterns, i.e. the number of mm of migration towards the nearest well containing attractant was measured.

Bactericidal test. Phagocytosis and bactericidal capacity of PMNL were tested in the phagocytosis system of Maaloe, as modified by Quie *et al.* (1967). The PMNL were suspended in Hank's balanced salt solution to a concentration of 10^7 per ml and exposed to 8-MOP and/or UV-A while kept in plastic tubes. The cells were then incubated in the presence of *Staphylococcus aureus* 502 A opsonized with pooled human serum. The fraction of bacteria remaining viable was then measured by counting the number of bacteria after a 24 h period in optimal growth conditions.

Lymphocyte proliferation. MMNL suspensions containing 3×10^6 lymphocytes per ml of culture medium were prepared as described. 8-MOP was added and 100 μl of the cell suspension was put into each well of a microtitre plate (Linbro Scientific Inc., Hamden, Conn., U.S.A.) before exposure to UV-A. One hundred μl of culture medium containing phytohaemagglutinin (PHA) (Wellcome Ltd, London, England) to give a final concentration of 2·5 μg/ml, or Cowan I strain of *Staphylococcus aureus* (Forsgren, Svedjelund & Wigzell, 1976) to give a final concentration of 1/1000 (50 bacteria per lymphocyte), was then added to each well. The lymphocytes were incubated for 3 days in 5% CO_2 at 37°C. [^{3}H]thymidine (New England Nuclear, Boston, Mass., U.S.A.) (40–60 Ci/mmol, final concentration 2 μCi/ml) was present during the last 24 h of the culture. Cultures were run in triplicate at each exposure dose and were harvested on to glass fibre filters using a Skatron harvesting machine (Skatron A/S, Lierbyen, Norway). The filters were dried and transferred to scintillation vials containing 4 ml of Luma Gel solution (Lumac B.V., Schaesberg, Netherlands). The radioactivity was measured in a Packard Tri-Carb liquid scintillation counter, and data were calculated from the mean of the triplicate cultures and expressed as percentages of the untreated controls.

Trypan blue viability test. Cells incubated at 37°C for 24 h after treatment with 8-MOP and/or UV-A were mixed with trypan blue (Flow Labs, U.S.A.) (0·5% in 0·85% saline). A few minutes later the relative frequency of dye-excluding (viable) cells was determined by microscopic inspection.

Statistical analysis. Student's *t*-test was used for the data of Table 1. Values of exposed and untreated cells obtained within one and the same experiment were compared, in order to exclude any influence due to inter-experimental variation.

RESULTS

Chemotaxis

Human PMNL and MMNL, respectively, were isolated *in vitro*, treated with 8-MOP and/or UV-A radiation, and finally analysed for their ability to migrate under agarose (Table 1). Within about 3 h PMNL were found to have left their initial zone, whereas the MMNL did not show a similar degree of migration until after about 24 h. Pretreatment of the leukocytes with UV-A alone (up to 2 J/cm^2) did not appreciably affect the results, whereas at a high concentration of

TABLE 1. Chemotaxis of human leukocytes pretreated with 8-MOP and/or UV-A. Migration distance in per cent (untreated cells = 100) ± standard error is shown[a]

Type of leukocyte tested	8-MOP (μg/ml)	UV-A (J/cm²)			
		0	0·1	0·3	2·0
Monomorphonuclear	0	100	n.d.	n.d.	86 ± 18
	1	105 ± 29	126 ± 11	142 ± 36	6 ± 6[b]
	20	100 ± 18	n.d.	1 ± 1[b]	0[b]
Polymorphonuclear[c]	0	100	102 ± 4	100 ± 4	85 ± 5
	1	88 ± 9	90 ± 4	91 ± 3	83 ± 8
	20	53 ± 3[b]	54 ± 3[b]	52 ± 4[b]	40 ± 6[b]

[a] For monomorphonuclear cells four to eight determinations per exposure dose were made, and for polymorphonuclear cells nine to ten determinations.

[b] $P < 0{\cdot}001$. Student's *t*-test.

[c] No statistical difference was noted between psoralen-treated and PUVA-exposed polymorphonuclear cells.

psoralen alone (20 μg/ml) the distance migrated by the PMNL was diminished ($P < 0{\cdot}001$). With the MMNL, some of the higher PUVA doses (i.e. 1 μg/ml of 8-MOP plus 2 J/cm² of UV-A and 20 μg/ml plus 0·3–2 J/cm²) led to a clear reduction of the migrated distance ($P < 0{\cdot}001$), whereas at the other PUVA doses the observed values were similar to those of the untreated MMNL. With the PMNL, on the other hand, no influence due to the combination of 8-MOP and UV-A on the migration could be revealed, since the inhibitory effect caused by 20 μg/ml of 8-MOP was not potentiated by exposure to UV-A. Experiments with PUVA-treated MMNL were regularly made in parallel to these PMNL determinations, precluding inactivity of the 8-MOP and/or the UV-A source employed.

In the experiments using MMNL suspensions only a minority of the cells, characterized morphologically by a horseshoe-shaped nucleus and therefore judged most likely to be macrophages, were seen to migrate. Most of the cells were morphologically typical lymphocytes which were not observed to migrate. However, at the highest PUVA dose (20 μg/ml of 8-MOP plus 2 J/cm² of UV-A) almost all of the cells, irrespective of their morphology, were found to have left their well. This is in contrast to controls and all lower exposure doses where only macrophage-looking cells could be observed to migrate. The migration of cells exposed to the highest PUVA dose was therefore not considered to represent true chemotaxis; this phenomenon may perhaps be due to toxic degradation of membranes.

Phagocytosis and killing of bacteria

In the experiments of Figs 1 and 2, PMNL were treated with 8-MOP and/or UV-A and then incubated for 30, 60 or 120 min in the presence of Staphylococci at a fixed leukocyte/bacteria ratio. After these different times killing of bacteria was determined. At some of the higher 8-MOP concentrations (10 μg/ml and upwards; clinical blood concentration is around 0·1 μg/ml), a dose-dependent decrease in the bactericidal function was observed (Fig. 1). The only additive effect due to UV-A, when combined with 8-MOP, was a minor increase in the percentage of surviving bacteria at a very high dose level (20 μg/ml plus 2 J/cm², see Fig. 2). No

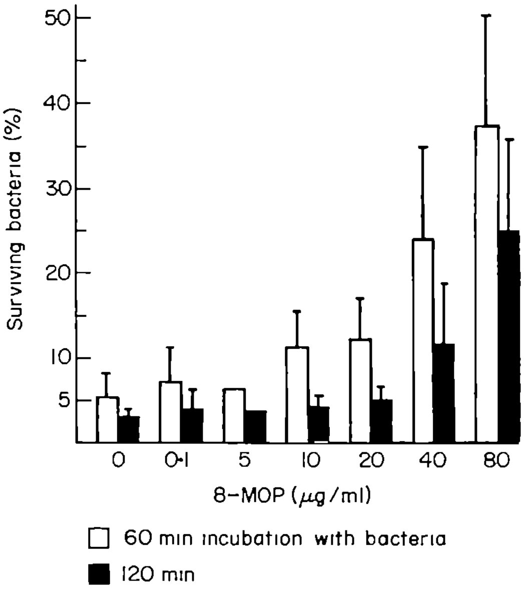

FIGURE 1. Phagocytosis and bactericidal action of human polymorphonuclear leukocytes treated with 8-MOP alone. Mean values are based on 2 to 11 determinations and s.e. is included at points with three or more determinations.

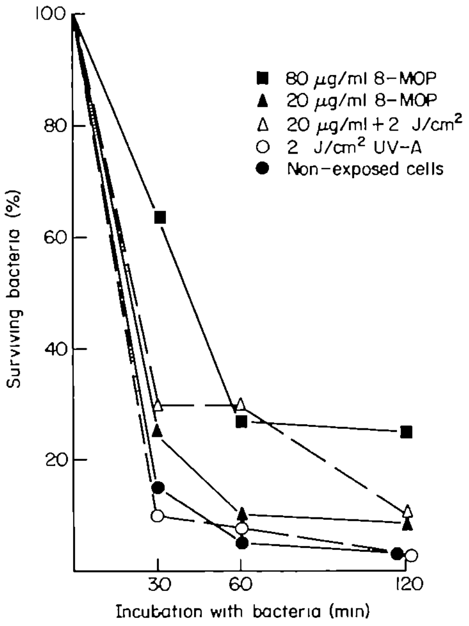

FIGURE 2. Phagocytosis and bactericidal action of human polymorphonuclear leukocytes exposed to 8-MOP and/or UV-A. One representative experiment.

influence due to exposure to UV-A radiation alone (doses between 0·03 and 8 J/cm^2; results not shown except for 2 J/cm^2 in Fig. 2) could be revealed.

Lymphocyte proliferation

MMNL suspensions containing 8-MOP were pipetted into wells of microtitre plates. Cells in one and the same plate were then exposed to UV-A for different times, at the end of which the appropriate wells were covered with a sheet of UVA-opaque black paper. PHA or *S. aureus* Cowan I was added in order to induce lymphocyte proliferation and cultures were run for 72 h. $[^3H]$thymidine was present during the last 24 h before the amount of incorporated radioactivity was measured. In Fig. 3 it can be seen that UV-A radiation or 8-MOP alone (highest concentration tested was 20 μg/ml) did not appreciably affect the results. At 0·1 μg/ml of 8-MOP (similar to the reported level of the clinical blood concentration) there was a gradual, UV-A dose-dependent reduction of thymidine incorporation beginning at 0·1 J/cm^2 (similar to the UV-A dose estimated to reach the circulating blood cells during PUVA treatment *in vivo*). When the 8-MOP concentration was raised to 1 μg/ml the inhibition of lymphocyte proliferation became clear already at 0·03 J/cm^2, and was nearly total at about 1 J/cm^2 (Fig. 3). Almost identical results were obtained when *S. aureus* Cowan I was used as mitogen instead of PHA (only some doses of Fig. 3 were tested). This indicates that B lymphocytes (reported to be stimulated by *S. aureus* Cowan I) show a similar sensitivity to PUVA as the T-lymphocyte population stimulated by PHA.

Effects of psoralen–DNA monoadducts and cross-links

In the experiments of Table 2 a second UV-A dose was administered to PUVA-treated and carefully washed cells in order to photoactivate free ends of psoralen–DNA monoadducts and thereby convert some of these lesions into cross-links. With human skin fibroblasts and Chinese hamster cells it has been shown that effects resulting from a second UV-A dose are dependent on

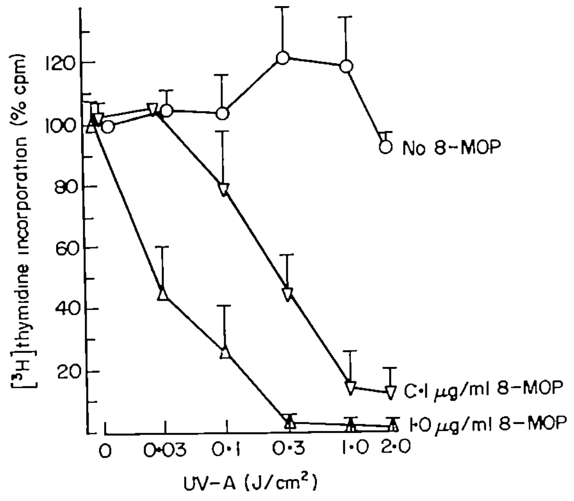

FIGURE 3. $[^3H]$thymidine incorporation in human PHA-stimulated lymphocytes treated with 8-MOP and/or UV-A. The values of per cent c.p.m. (control = 100) are based on 2 to 7 separate determinations in triplicate, and s.e. is shown where at least three determinations were made.

TABLE 2. Effect of PUVA plus a second UV-A dose on [^{3}H]thymidine incorporation in PHA-stimulated human lymphocytes[a]

	Treatment of cells[b]				
Type	8-MOP (μg/ml)	UV-A before wash (J/cm^2)	UV-A after wash (J/cm^2)	[^{3}H]thymidine incorporation (c.p.m. × 10^{-3})	Possible interaction between UVA-activated psoralen and DNA
A	0·1	—	—	51	None
B	0·1	—	2·0	45	'Wash-resistant' 8-MOP is bound to DNA (monoadducts and cross-links are formed)
C	0·1	0·1	—	24	Psoralen–DNA monoadducts and cross-links are formed
D	0·1	0·1	2·0	18	Effects of the second UV-A dose: 1. 'Wash-resistant' 8-MOP is bound to DNA 2. Monoadducts formed during the first UV-A exposure are converted into cross-links
E	0·1	2·0	—	0·2	Psoralen–DNA monoadducts and cross-links are formed (positive control exposure)

[a] Results of one representative experiment (total five) are shown.

[b] An extensive wash (see Methods section) was part of all types of treatment.

DNA cross-links rather than on monoadducts (Ben-Hur & Elkind, 1973; Hanawalt, Liu & Parsons, 1981; Bredberg, 1983). Lymphocytes were treated with 8-MOP and/or UV-A and then cultivated as described for Fig. 3. To the best of our knowledge no data on psoralen damage to human lymphocyte DNA have been reported. However, since DNA cross-links have been found in human lymphoblastoid cells exposed to low PUVA doses (Cohen, L.F. *et al.*, 1981), it seems likely that PUVA plus a second UV-A dose will increase the fraction of cross-linked DNA also in human lymphocytes. The lower amount of incorporated thymidine observed after treatment type B, as compared with type A, indicates that the washing procedure cannot fully deplete the cell suspensions of unbound 8-MOP. Instead some residual 8-MOP must still have been present after the wash to become UVA-activated and linked to DNA. A PUVA dose of 0·1 μg/ml plus 0·1 J/cm^2 (treatment type C) gave rise to a partial inhibition which was comparable to that noted in Fig. 3 where no wash was employed. Addition of a second UV-A dose to treatment type C resulted in a further reduction of the incorporation rate (treatment type D). This decrease (from 24 to 18) was small and similar to that caused by the UV-A dose of treatment type B (from 51 to 45). It therefore seems as if the effect exerted by the second UV-A dose of treatment type D was mediated mainly by the binding of free and 'wash-resistant' 8-MOP molecules to DNA. Therefore, a conversion of monoadducts, preformed during the initial PUVA treatment, into cross-links obviously added very little to the depression of the incorporation rate induced by treatment type D.

Trypan blue dye exclusion test of viability

MMNL and PMNL, respectively, were treated with 8-MOP and/or UV-A at all doses indicated in Table 1 and then incubated at 37°C for 24 h before their ability to exclude trypan blue was determined. Between 85% and 98% of the cells excluded the dye, except for the PMNL treated with the highest PUVA dose (20 μg/ml of 8-MOP plus 2 J/cm^2 of UV-A), where a clear decrease in the relative frequency of excluding cells was observed (data not shown).

DISCUSSION

Among the present studied human leukocyte functions only lymphocyte proliferation was clearly affected by low doses of PUVA (similar to that dose which, on average, each of the peripheral circulating blood cells is estimated to be exposed to during one PUVA treatment session *in vivo*). No influence of clinically relevant doses of 8-MOP or UV-A *per se* was noticed. These results are in agreement with the observations that *in vivo* PUVA therapy appreciably affects functions of T lymphocytes in contrast to those conducted by PMNL (Kraemer, 1982; Silny *et al.*, 1980).

The experiments with PUVA containing a split UV-A dose did not provide any evidence that cross-links depress lymphocyte proliferation more efficiently than monoadducts (see Table 2). Previous studies have shown that cross-links exert a pronounced lethal effect and decrease the relative number of cells reaching mitosis after PUVA treatment (Bredberg, 1983; Jabłońska *et al.*, 1982). These studies, indicating that cross-links are strongly cytotoxic, obviously are in conflict with our present data on lymphocyte proliferation. However, it has been shown that PUVA-treated cells are not halted until they reach the G$_2$ phase (situated between DNA synthesis and mitosis) (Cohen, S.R. *et al.*, 1981). Therefore it is possible that the cytotoxic action of psoralen–DNA cross-links is characterized by a post-replication arrest which would be expected to lead to a normal rate of thymidine incorportion during the first cell cycle after PUVA treatment, but to a decreased frequency of cells in mitosis.

The ability of the presently investigated PMNL functions to withstand high dose PUVA treatment does not seem to depend primarily on a lower cellular sensitivity *per se* to PUVA, since PUVA-exposed MMNL were at least as proficient in excluding trypan blue as identically treated PMNL. Instead, a critical factor for the studied cell functions to become affected by PUVA seems to be the length of time between PUVA exposure and analysis, rather than the type of leukocyte.

It has been reported that *in vivo* PUVA treatment of guinea-pigs primarily causes a depression of DNA synthesis, whereas RNA and protein synthesis do not become affected until 1–3 days later (Epstein & Fukuyama, 1975). A key role of DNA as a target for PUVA therapy has been indicated by studies on the mixed leukocyte reaction (Kraemer, 1982). Responder cells (defined as proliferating cells) were then noted to be more sensitive to PUVA than stimulator cells (defined as antigen-presenting cells), suggesting that DNA damage, as compared with effects on membrane-dependent functions, occurs at a relatively low PUVA dose level. However, membrane damage has been observed in human cells exposed to PUVA *in vitro* (Wennersten, 1979). This type of damage, by leading to immune disturbance, has been suggested to be of greater importance for effects of clinical PUVA therapy than DNA alterations (Morhenn, Benike & Engleman, 1980).

Our present finding that a time lag between PUVA exposure and analysis of cell function better demonstrates the sensitivity of human leukocytes to PUVA gives support to the view that biological effects of low dose PUVA are mainly due to the formation of psoralen adducts in DNA. Of course this view does not preclude that PUVA-induced immunological effects can be mediated by changes in other cell components than DNA, since a disturbance of the information encoded within DNA may be transmitted through RNA and protein synthesis to cause finally an alteration in, for example enzymes and membrane antigens.

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In vitro complement activation by antinuclear antibodies in the epidermis from patients with systemic sclerosis

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SUMMARY

Skin biopsies from twenty-one systemic sclerosis patients with significant serum fluorescent antinuclear antibody (FANA) titres (more than 1:40) were examined by direct immunofluorescence (DIF) and by *in vitro* immunofluorescent complement (C) activation (C+DIF). Eight patients showed epidermal nuclear ANA deposits by DIF. *In vitro* complement activation was achieved in epidermal nuclei from all eight patients. In addition, one patient with diffuse scleroderma revealed a dense speckled, and two patients with the CREST syndrome a discrete speckled, epidermal nuclear staining pattern by C+DIF. The latter two patients showed high titres of anticentromere antibody in their sera. We consider that the C+DIF findings of these two patients with the CREST syndrome reflect binding of serum anticentromere antibodies to their antigens *in vivo*.

The presence of epidermal nuclear antinuclear antibody (ANA) deposits in skin biopsies from patients with systemic lupus erythematosus (SLE) and mixed connective tissue disease (MCTD) who have high titres of serum ANA against nuclear ribonucleoprotein (n-RNP) or Sm-antigen is a well-described phenomenon (Tan & Kunkel, 1966; Gilliam, Smiley & Ziff, 1975; Wells *et al.*, 1979; Bentley-Phillips & Geake, 1980; Kallenberg *et al.*, 1983).

IgG-class antibodies to n-RNP are capable of penetrating viable blood mononuclear cells via the Fc-receptor for immunoglobulins (Alarcón-Segovia, Ruiz-Argüilles & Fishbein, 1978). The mechanism of ANA penetration into living epidermal cells, however, is unknown. Although controversial (Iwatsuki *et al.*, 1982), there is evidence to support the *in vivo* occurrence of epidermal nuclear ANA in patients with serum antibody to n-RNP (Izuno, 1978; Wells *et al.*, 1979; Gallopin & Saurat, 1981).

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Recently, we demonstrated that epidermal ANA deposits also occur in systemic sclerosis patients with serum ANA specificities different from n-RNP (Reimer *et al.*, 1983).

In the present study, the *in vitro* immunofluorescent complement activation method was applied to skin biopsies from patients with systemic sclerosis. The scope of this study was to evaluate further the phenomenon of epidermal nuclear ANA in these patients.

METHODS

The patients selected for this study demonstrated significant (> 1:40) serum FANA titres. They were divided into two groups. Group 1 consisted of fourteen patients (twelve women and two men) with at least four features of the CREST syndrome (calcinosis, Raynaud's phenomenon, oesophageal dysmotility, sclerodactyly, telangiectasia). Their mean age was 52 years with a range from 34 to 78 years. The mean duration of disease was 11 years, ranging from 1 to 28 years. Group 2 included seven patients with diffuse scleroderma. There were three women and four men, with a mean age of 49 years, and a range from 30 to 59 years. The mean duration of disease was 6 years ranging from 1 to 11 years.

Punch biopsies (4 mm) from the inner aspect of the upper arm were immediately frozen in liquid nitrogen, stored at −70°C and processed within 1 week. Specimens were sectioned (4–6 μm) in a cryostat and fixed in acetone for 3 min in the established immunofluorescence staining procedure (Beutner, Chorzelski & Bean, 1979). Fluorescent serum ANA titres and staining patterns for IgG were assessed using KB cells (Electro-Nucleonics, Bethesda, U.S.A.) as the substrate. *In vitro* immunofluorescent complement activation was performed according to the method described by Jordon, Sams & Beutner (1969). Skin cryostat sections were incubated for 30 min at 37°C with fresh normal AB serum (NHS) as a complement source at a 1:10 dilution in isotonic veronal-buffered saline (VBS) with $1{\cdot}5 \times 10^{-4}$ M $CaCl_2$ and $5{\cdot}0 \times 10^{-4}$ M $MgCl_2$, pH 7·4. The slides were washed in phosphate-buffered saline (PBS), pH 7·4, for 30 min with three changes of the buffer. As a control, skin sections from the same biopsy were incubated with VBS as well as heat-inactivated NHS instead of NHS. Complement activation was assessed by immunofluorescent C3c staining. To assess the ability of serum ANA to bind to epidermal nuclear antigens *in vitro*, and to activate complement, normal skin cyrostat sections were incubated with the patients' sera at 1:40 dilution for 30 min, rinsed in PBS and further processed by indirect immunofluorescence as well as the C+DIF technique. FITC-labelled monospecific goat antisera to human IgG, IgA and IgM (Tago, Inc., Burlingame, U.S.A.) and rabbit antisera to human C3c (Behringwerke AG, Germany) were used. FITC fluorescence was detected with a Leitz Orthoplan microscope, a Hg 50 W lamp, a Leitz Ploempak 2·1 vertical illuminator, filter-block I2 and NPL Fluortar oil immersion objectives.

RESULTS

The immunofluorescence findings and clinical data of the individual patients are shown in Table 1.

Direct immunofluorescence on skin biopsies from twenty-one systemic sclerosis patients revealed epidermal nuclear ANA deposits of IgG class in four cases with the CREST syndrome and in four patients with diffuse scleroderma. Additional speckled IgM was observed in a patient with diffuse scleroderma and IgA in a patient with the CREST syndrome.

The C+DIF technique used on skin sections from the above patients revealed complement activation (C3c) in all eight cases with a positive skin biopsy ANA test. In addition, one patient

TABLE 1. Clinical data and immunofluorescent findings in patients with systemic sclerosis

Case	Sex	Age (years)	Duration of symptoms (years)	Scleroderma variant	FANA titre, (pattern for IgG)	n-RNP† specificity	Epidermal nuclear Ig deposits			*In vitro* fluorescent complement activation C3
							IgG	IgA	IgM	
1	F	56	9	CREST	4096 (nu)	neg.	+	−	−	+ +
2	F	55	3	CREST	8192 (ls)	neg.	+ +	−	−	+ + +
3	F	34	1	CREST	4096 (nu)	neg.	+ +	−	−	+ + +
4	F	42	5	CREST	2048 (ce)	neg.	−	−	−	+ +
5	F	57	10	CREST	1024 (sp)	neg.	+	−	+	+ +
6	F	42	20	CREST	8195 (ce)	neg.	−	−	−	+ +
7	F	65	25	CREST	2048 (sp)	neg.	−	−	−	−
8	F	52	11	CREST	2048 (sp)	neg.	−	−	−	−
9	F*	54	16	CREST	512 (sp)	neg.	−	−	−	−
10	M	36	4	CREST	128 (sp)	neg.	−	−	−	−
11	F	54	16	CREST	512 (sp)	neg.	−	−	−	−
12	F	78	5	CREST	1024 (sp)	neg.	−	−	−	−
13	F	60	28	CREST	2048 (ce)	neg.	−	−	−	−
14	M	39	5	CREST	1024 (ce)	neg.	−	−	−	−
15	M	57	3	diffuse	2048 (sp)	neg.	+	−	−	+ +
16	M	38	2	diffuse	4096 (nu)	neg.	+ +	−	−	+ +
17	M	58	3	diffuse	2048 (sp)	neg.	+ +	+	−	+ + +
18	F	57	10	diffuse	2048 (sp)	neg.	−	−	−	+ +
19	M*	59	1	diffuse	2048 (sp)	neg.	+	−	−	+ + +
20	F	46	11	diffuse	1024 (sp)	neg.	−	−	−	−
21	F	30	11	diffuse	1024 (sp)	neg.	−	−	−	−

sp = dense speckles; ls = large speckles; nu = nucleolar; ce = centromere (discrete speckles).

* Patients receiving immunosuppressive treatment.

† Assessed by counter-immunoelectrophoresis (Institute of Immunology, University of Heidelberg, Germany).

with diffuse scleroderma and two patients with the CREST syndrome who were negative by DIF revealed complement C3c deposits by the C + DIF procedure. The patient with diffuse scleroderma showed a dense speckled and the patients with the CREST syndrome a discrete speckled epidermal nuclear staining pattern by C + DIF method. In the latter patients' epidermis, discrete speckles lined up in parallel were seen in the basal cells, possibly representing dividing keratinocytes (Fig. 1). The serum FANA patterns of these two patients showed the centromere staining on KB-cells.

When the patients' sera were overlaid on to normal human skin cryostat sections and processed by indirect immunofluorescence, there was binding of all the 21 different serum ANA to epidermal nuclei. These ANA were capable of activating complement *in vitro* as shown in a parallel incubation with complement and subsequent C3c immunofluorescent staining. The ANA patterns obtained by both procedures were identical, correlating with the FANA results obtained on KB-cells as the substrate and with epidermal nuclear ANA staining as observed by DIF and C + DIF in some patients' skin biopsies.

As a rule, the intensity of epidermal nuclear immunofluorescence by C + DIF was greater than by DIF alone and the reading more distinct.

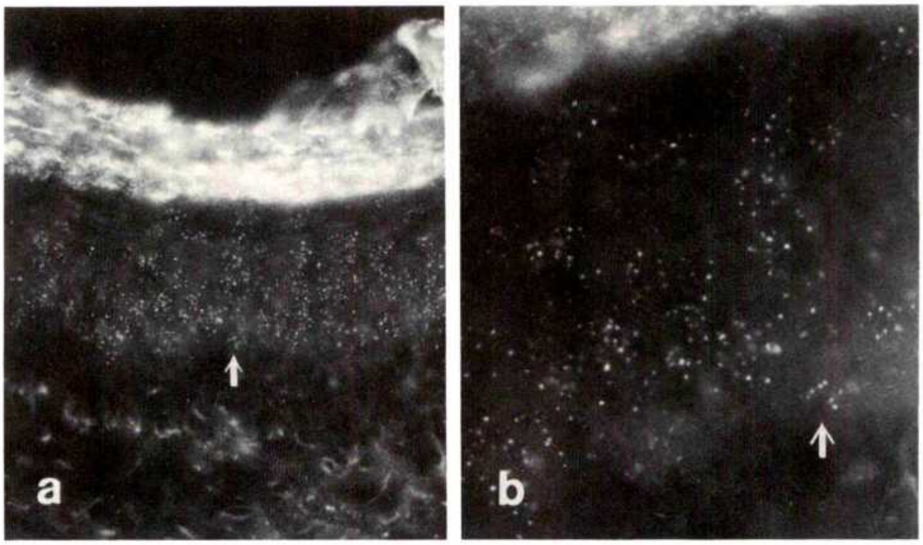

FIGURE 1. Immunofluorescent *in vitro* complement activation. Discrete speckled epidermal nuclear complement (C3c) in the skin biopsy from a patient with the CREST syndrome and serum anticentromere antibodies. Note alignment of fluorescent speckles in a parallel fashion of a basal cell (arrows), possibly a dividing keratinocyte (a and b). Also note stratum corneum immunofluorescence indicating the presence of complement fixing anti-stratum corneum antibodies (Binder, Beutner & Jablonska, 1980). (a) × 312·5; (b) × 1250.

DISCUSSION

In previous studies, epidermal nuclear ANA from patients with SLE or MCTD were found to activate complement *in vitro* (Andersen *et al.*, 1983; Shu *et al.*, 1977). These patients revealed serum ANA either to Sm or n-RNP or both nuclear antigens as a common serological finding. Direct immunofluorescence, however, has so far failed to show complement components in epidermal nuclei suggesting that epidermal ANA, though capable of binding complement, do not activate the complement cascade *in vivo*.

In a recent study using DIF, we reported on the phenomenon of ANA in skin biopsies from scleroderma patients without the n-RNP specificity of serum ANA (Reimer *et al.*, 1983). Among these ANA were those giving either a large coarse, a dense fine, a thready or a nucleolar staining pattern by indirect immunofluorescence on KB-cells as the substrate. We failed to detect epidermal nuclear immunoglobulin deposits in patients with the CREST-syndrome who revealed circulating anticentromere antibodies. We were also unable to detect complement in epidermal nuclei from those patients with a positive skin biopsy ANA test.

In the present study, *in vitro* complement activation was detected in epidermal nuclei from all scleroderma patients with epidermal nuclear ANA by direct immunofluorescence. In addition, three patients who were negative by DIF revealed complement activation in epidermal nuclei by the C+DIF technique. One patient with diffuse scleroderma showed a dense speckled and the others a discrete speckled epidermal nuclear staining by C+DIF. The latter patients had features of the CREST syndrome. Indeed, the FANA test on KB-cells showed the centromere staining pattern which is highly characteristic of the CREST syndrome (Tan, 1982). Though undetectable by direct immunofluorescence, we conclude that serum anticentromere antibodies in these two patients may have reacted with their antigens *in vivo*, thus accounting for complement activation *in vitro* as shown by immunofluorescent C3c staining.

It is noteworthy that epidermal nuclear ANA staining patterns in skin biopsies of the individual patients always correlated with the FANA patterns obtained on control skin sections and on KB-cells as the substrate.

Immunofluorescent complement activation *in vitro* is a sensitive method for detection of complement binding antibodies in tissue sections or cells (Chorzelski *et al.*, 1983; Jordon *et al.*, 1969). Since each antibody molecule can trigger the activation of hundreds of complement molecules, a considerable amplification of the antigen–antibody reaction can be achieved which otherwise may have escaped detection by direct immunofluorescence. Indeed, the brightness of immunofluorescence and the clarity of reading by the C + DIF method exceeded the results obtained by the DIF technique in most cases.

Thus, the sensitivity of immunofluorescent complement activation *in vitro* can explain detection of anticentromere antibodies in skin biopsies from the two patients with the CREST syndrome.

In SLE and MCTD, a dysregulatory effect of intracellular anti-DNA and anti-nRNP antibodies on the cell cycle of Fc-receptors bearing T cells with loss of function was recently demonstrated (Alarćon-Segova & Llorente, 1983). The significance of *in vivo* epidermal nuclear ANA in systemic sclerosis and other connective tissue diseases is unknown and requires further investigation.

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Expression of differentiation antigens in benign sweat gland tumours

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SUMMARY

Fifty benign sweat gland tumours were studied for the expression of carcinoembryonic antigen (CEA) and apocrine epithelial antigen (AEA), using immunohistochemical methods. CEA was found in thirty-two and AEA in thirty-three neoplasms. Both antigens were located in the epithelium of the luminal structures and in the intraluminar material and CEA was occasionally found also in proliferating cells. Co-expression of CEA and AEA occurred frequently in cases of syringoma, syringocystadenoma papilliferum, hidradenoma papilliferum, eccrine spiradenoma and clear cell hidradenoma. AEA was seen also in tumours showing eccrine differentiation, even though it is not present in normal eccrine sweat ducts.

Human sweat and the epithelium of eccrine and apocrine sweat glands and ducts contain carcinoembryonic antigen (CEA) (Penneys, Nadji & McKinney, 1981) which was originally thought to occur only in fetal and neoplastic epithelial cells of the large bowel (Gold & Freedman, 1965). Using antiserum against human milk fat globule protein, an apocrine epithelial antigen (AEA) has been shown in the epithelium of mammary, sebaceous and apocrine sweat glands (Ceriani *et al.*, 1977; Heyderman, Steele & Ormerod, 1979). We have recently shown that the Paget cells in mammary and extramammary Paget's disease frequently contain both CEA and AEA (Kariniemi *et al.*, 1984). This finding prompted us to investigate the expression and localization of these antigens in sweat gland tumours using immunohistochemical methods.

METHODS

Paraffin-embedded histological specimens of fifty benign sweat gland tumours were collected from the Central Laboratory of Pathology of the Helsinki University and from the Department

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of Dermatology, Helsinki University Central Hospital. The material has been detailed in Table 1.

Antisera

Rabbit anti-CEA serum was purchased from Dako Immunoglobulins (Copenhagen, Denmark) and was further absorbed with a perchloric acid extract of human spleen to ensure the specific reaction as described earlier (Lindgren, Wahlström & Seppälä, 1979). The preparation of rabbit anti-AEA serum has been described recently (Kariniemi *et al.*, 1984; Forsman *et al.*, 1984). Briefly, membranes from isolated human milk fat globules were separated by extraction with phosphate-buffered saline (PBS) and were solubilized in 1% Triton X-100 in PBS. The glycoproteins were isolated in a concanavalin A-sepharose column and eluted by alpha-methyl-pyranomannopyranoside. Rabbits were repeatedly immunized with formalin-treated glycoproteins emulsified in Freund's adjuvant and the resulting antisera were absorbed before use with erythrocytes and normal blood leukocytes from buffy coats of blood units of 400 ml (kindly provided by the Finnish Red Cross Blood Transfusion Service).

Immunoperoxidase and immunofluorescence staining

For immunoperoxidase staining the endogenous peroxidase activity of the deparaffinized histological sections was extinguished by treatment with methanol containing 0·3% hydrogen peroxide for 30 min at room temperature. The sections were washed in PBS containing 2% normal swine serum and incubated in moist chambers with 1:100 diluted anti-CEA or anti-AEA sera at 37°C for 60 min. After washing with PBS the sections were incubated with biotinylated swine anti-rabbit IgG (Vectastain®, ABC kit, Vector Laboratories, Inc., Burlingame, Calif., U.S.A.) at 37°C for 30 min, washed again and incubated for 30 min with Vectastain® ABC Reagent following the instructions of the manufacturer.

The colour reaction was developed by incubating the sections with a solution containing 0·04% 3-amino-9-ethyl-carbazole and 0·01% hydrogen peroxide in 0·05 mM sodium acetate buffer at 37°C for 15 min. Control sections were treated in a similar manner with either one or both antisera in advance, and fully absorbed with purified CEA or AEA, or the antisera were

TABLE 1. The results of immunostaining for CEA and AEA in different sweat gland tumours

		Number of positive cases	
Type of sweat gland tumour	Number	for CEA	for AEA
Syringoma	8	7	7
Syringocystadenoma papilliferum	7	6	7
Hidradenoma papilliferum	3	3	3
Clear cell hidradenoma	7	4	5
Eccrine spiradenoma	11	8	7
Cylindroma	5	2	2
Hidrocystoma	2	2	2
Eccrine poroma	5	0	0
Pleomorphic adenoma	2	0	0
Total	50	32	33

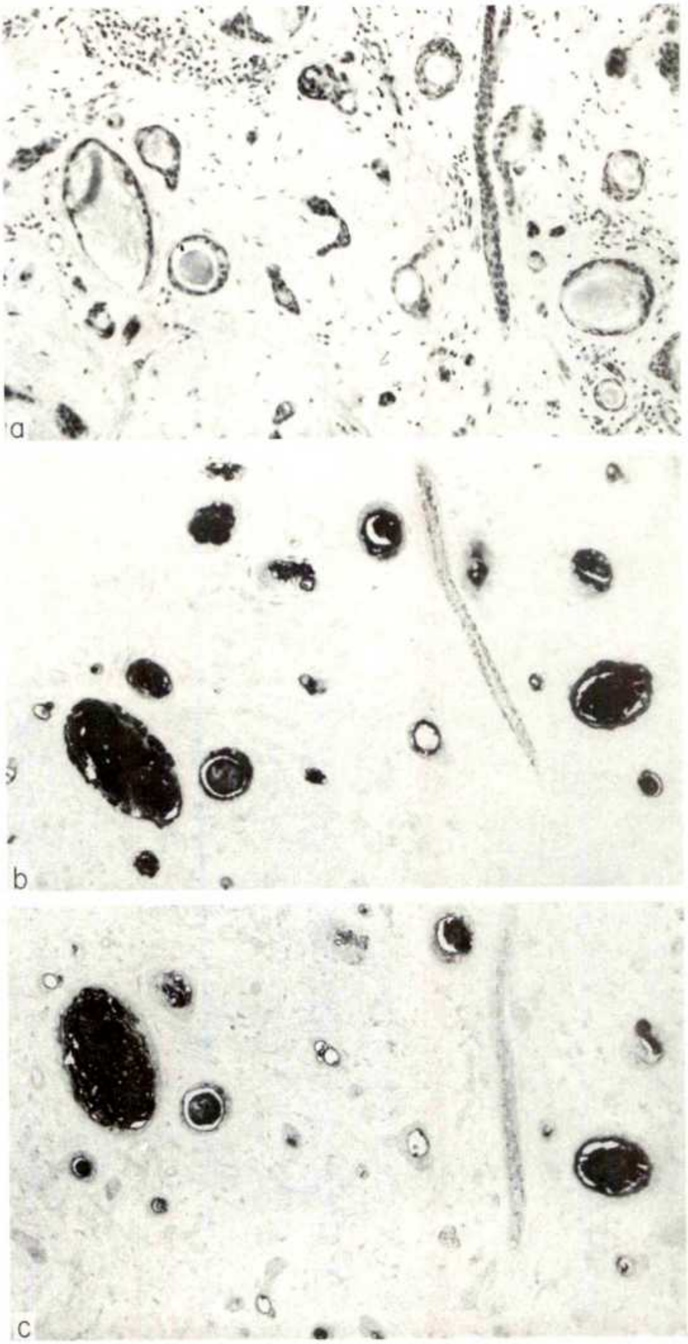

FIGURE 1. Syringoma. (a) Haematoxylin–eosin. (b) Immunoperoxidase staining for CEA shows a positive reaction in the epithelium of the cystic ducts, in the intraluminary material and in the epithelium of normal eccrine sweat duct. (c) Immunoperoxidase staining for AEA shows a co-expression with CEA. AEA is not seen in normal eccrine sweat duct. (Original magnification × 250.)

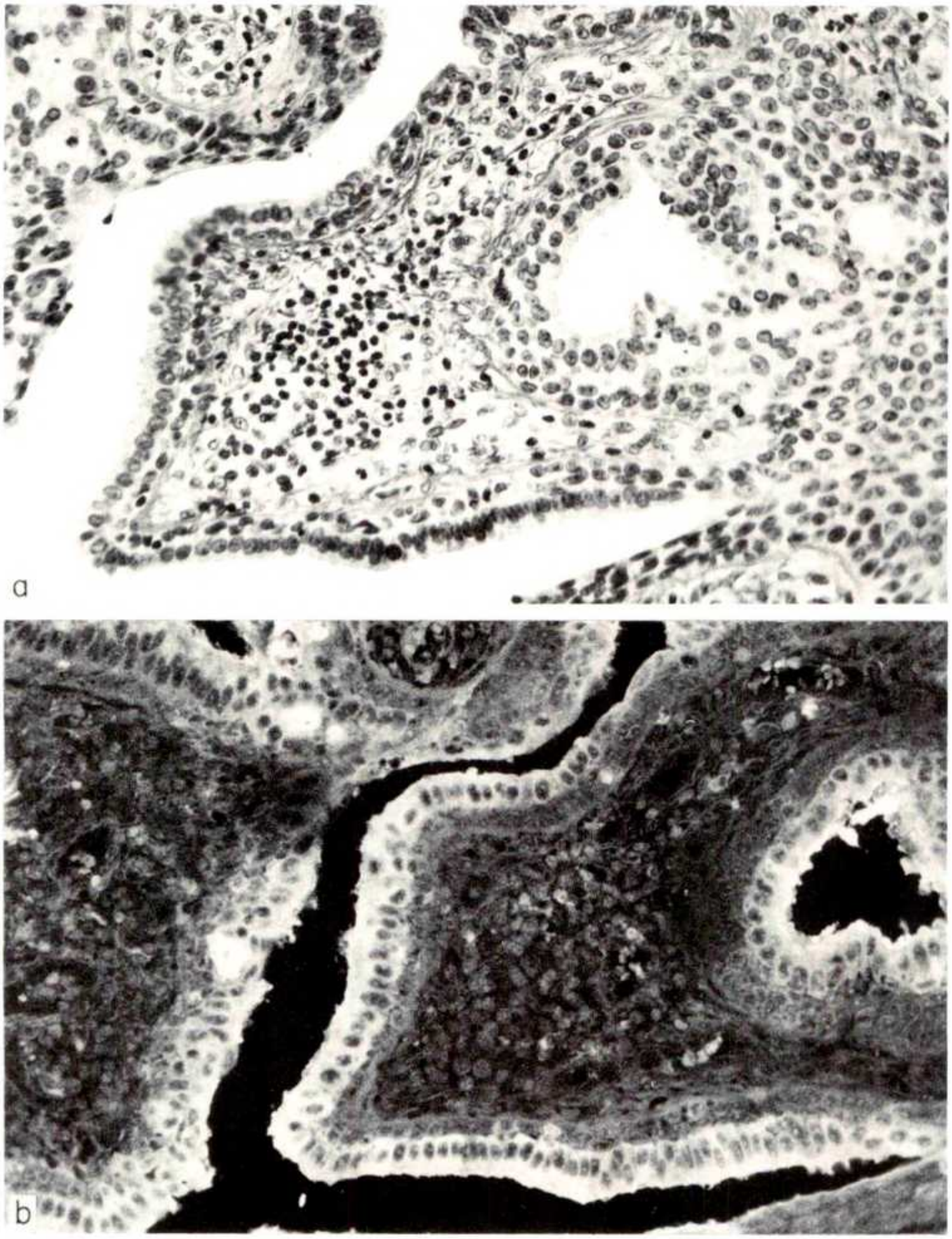

FIGURE 2. Syringocystadenoma papilliferum. (a) Haematoxylin–eosin. (b) Indirect immunofluorescence for AEA shows a positive reaction in the epithelium of the papillary projections. (Original magnification × 400.)

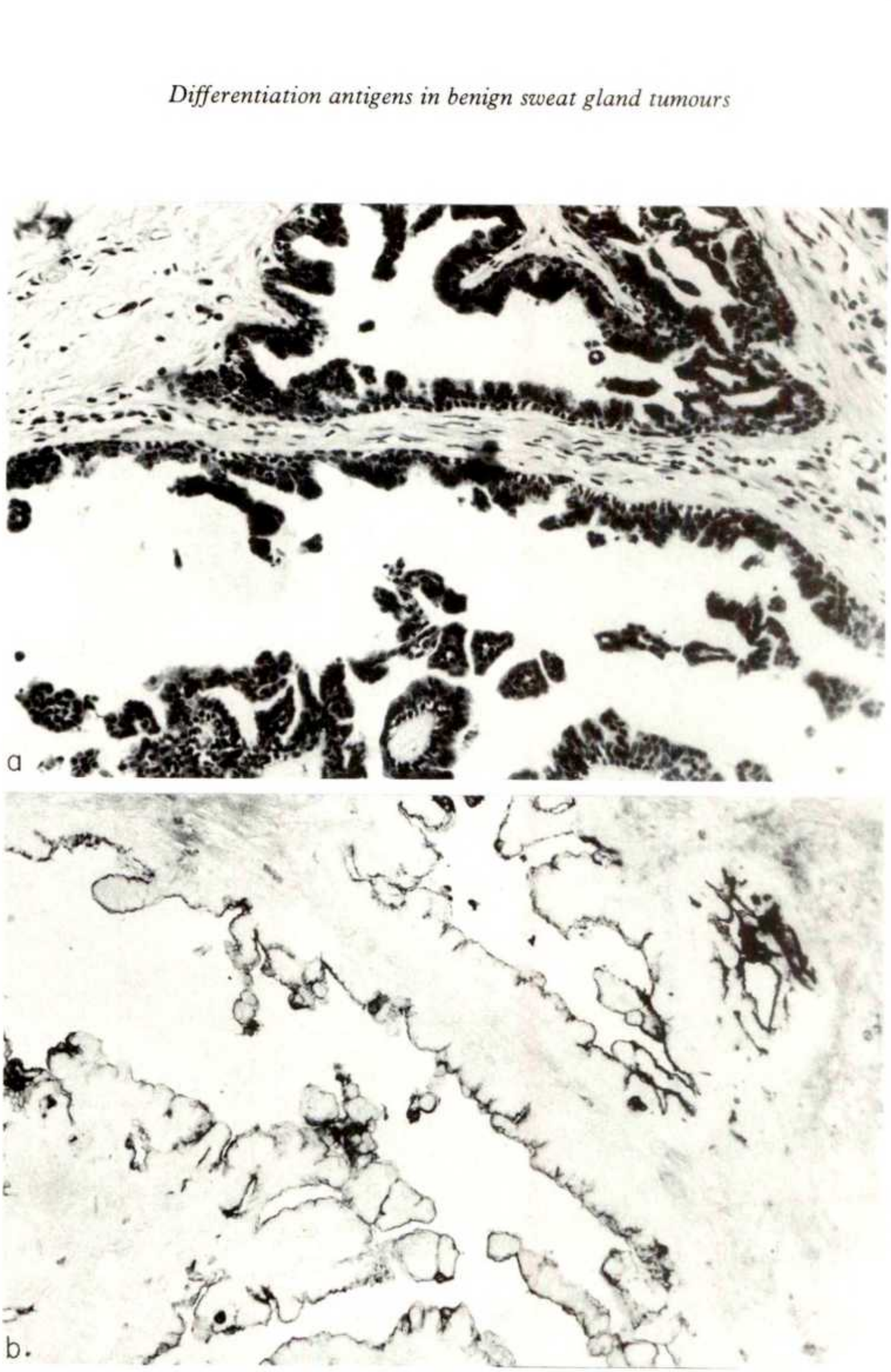

FIGURE 3. Hidradenoma papilliferum. (a) Haematoxylin–eosin. (b) Immunoperoxidase staining for AEA is positive at the luminal side of the epithelium. (Original magnification × 250.)

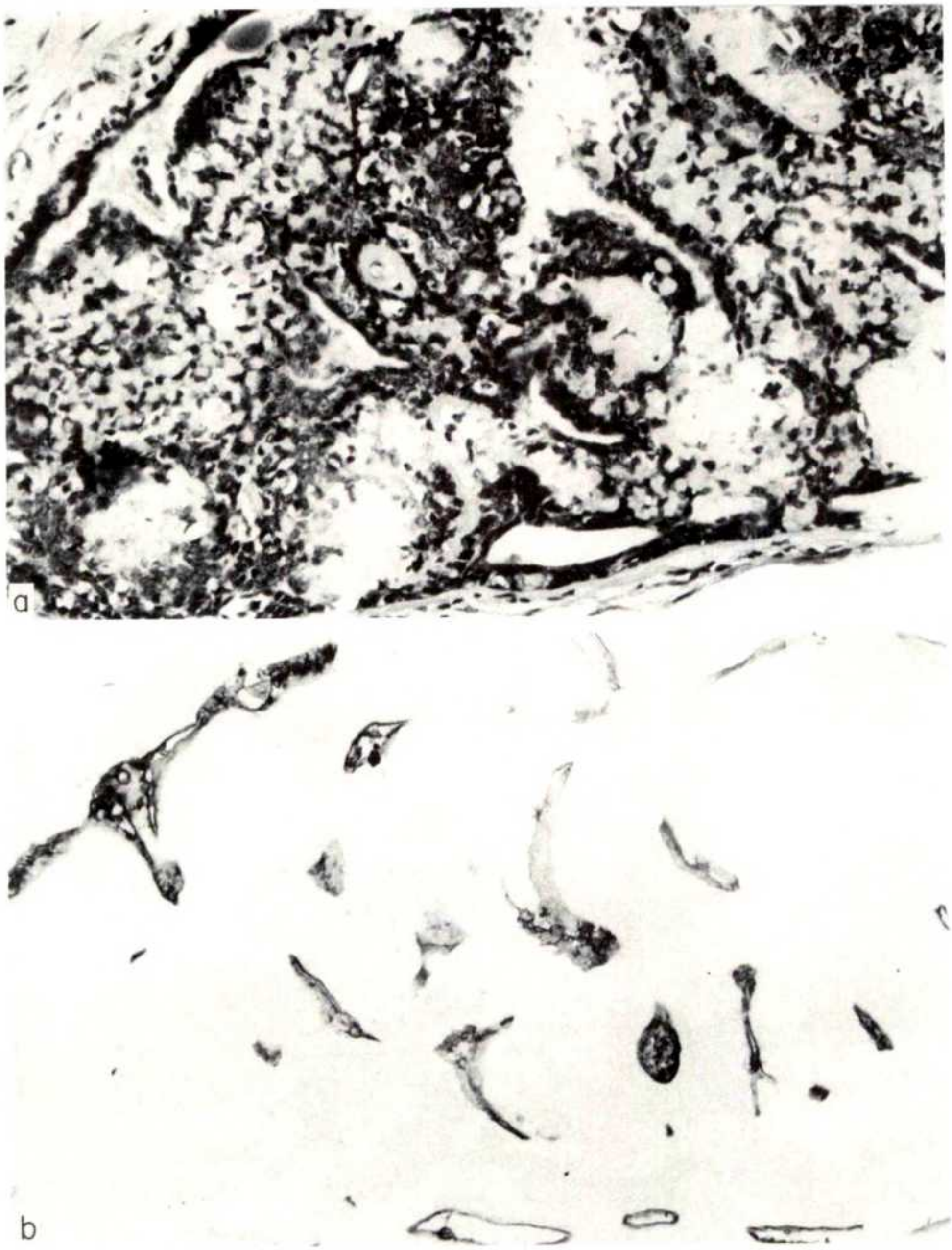

FIGURE 4. Eccrine spiradenoma. (a) Haematoxylin–eosin. (b) Immunoperoxidase staining for CEA is positive in the epithelium of the tubular lumina. (Original magnification × 250.)

replaced by normal rabbit serum or by PBS. In still other controls each of the following reagents were replaced with PBS. No counterstain was added to the immunoperoxidase stainings, but adjacent sections were stained with haematoxylin–eosin.

The expression of AEA in sweat gland tumours was also studied by indirect immunofluorescence. For immunofluorescence staining, deparaffinized histological sections were incubated for 45 min in a moist chamber with 1:20 diluted rabbit anti-AEA serum or preimmune rabbit serum. After washes with PBS containing 5% fetal calf serum the sections were treated for an additional 30 min with 1:30 diluted fluorescein isothiocyanate-conjugated swine anti-rabbit immunoglobulin (Dakopatts, Copenhagen, Denmark). The sections were washed as above and mounted in PBS–glycerol (pH 8).

RESULTS

The results of the immunostaining for CEA and AEA in different sweat gland tumours are shown in Table 1. Most of the tumours contained both of these antigens. Expression of AEA was similar using either the immunoperoxidase or the immunofluorescence method. Both CEA and AEA were predominantly found in the epithelium of the luminal structures and in the intraluminary secretion. Hence they were frequently found in the tumours containing tubular lumina. In syringomas the epithelial cells of small cystic ducts were strongly positive with anti-CEA and anti-AEA sera (Fig. 1a,b,c), except in one case where only solid strands of epithelial cells without luminal structures were seen. The staining intensity for AEA was, however, weaker than that for CEA (Fig. 1b,c). AEA was seen in all cases of syringocystadenoma papilliferum in the epithelium of papillary projections and in intraluminary secretion (Fig. 2a,b), whereas only six of these tumours contained CEA. In contrast to syringomas, the AEA-reaction was clearly stronger than the staining intensity for CEA in these tumours. The similar staining pattern was seen also in three cases of hidradenoma papilliferum, in which epithelial cells stained stronger for AEA than for CEA (Fig. 3a,b). In eccrine spiradenoma and clear cell hidradenoma, CEA and AEA were found only in some of the cases, where they were also located in the epithelium of tubular lumina of the tumours (Fig. 4a,b), whereas the proliferating cells in the cell islands only occasionally contained CEA, but not AEA. The expression of these antigens was similar in cylindromas, where CEA and AEA, if present, were found only in those tumours containing tubular lumina. However, CEA was sometimes found also in proliferating cells. The epithelial cells of two cases of hidrocystoma contained both CEA and AEA, whereas the tumour cells in eccrine poromas and pleomorphic adenomas were negative for both antigens in all cases studied.

DISCUSSION

Our results show that in benign sweat gland tumours, cells differentiating toward ductal structures frequently express both CEA and AEA, whereas cells showing glandular differentiation seldom contain these antigens. This finding is in line with the results of Penneys and his co-workers, who found a similar distribution of CEA in sweat gland neoplasms (Penneys, Nadji & Morales, 1982). In contrast to the results of Penneys *et al.* (1982), CEA was not present in all cases of a given type of neoplasm in our study. Especially in clear cell hidradenomas and in eccrine spiradenomas part of the tumours were totally negative for CEA, even if they all contained tubular lumina. The negative cases of cylindromas in our series contained few luminal structures and practically no lumina were seen in the cases of eccrine poroma, which were all negative for CEA. The variability in the expression of CEA in sweat gland tumours may be based on differences in the line and stage of differentiation in a single tumour.

AEA has been demonstrated in most organs showing glandular differentiation with apocrine secretion (Heyderman *et al.*, 1979). Thus apocrine sweat glands contain AEA, while eccrine sweat glands are virtually negative. The expression of this antigen may thus reflect apocrine differentiation of the tumour. In our series AEA was found in all cases of syringocystadenoma papilliferum and hidradenoma papilliferum, which are thought to represent apocrine adenomas with differentiation toward apocrine ducts and glands, respectively. Two cases of cylindromas, which also show differentiation toward apocrine structures, contained AEA. This antigen was also found in the flattened epithelium of two hidrocystomas in our study. According to this finding our tumours, although showing eccrine differentiation at light microscopy level, may in fact be apocrine hidrocystomas, in which the cyst wall lining is attenuated. However, we could demonstrate AEA also in sweat gland neoplasms with eccrine differentiation. Thus syringomas and part of the clear cell hidradenomas and eccrine spiradenomas also contained AEA in luminal structures, even if the staining intensity in these tumours was weaker than in tumours with apocrine differentiation.

Although the CEA-positive neoplasms usually also expressed AEA, some tumours in our series contained only one of these antigens. Differences were also seen in their staining intensity. These findings together with earlier results with absorbed antisera (Heyderman *et al.*, 1979) confirm the lack of identity between these antigens.

Adnexal tumours with pilar and sebaceous differentiation do not contain CEA (Penneys *et al.*, 1982), whereas sebaceous neoplasms express AEA, but not CEA (unpublished results). Demonstration of these antigens in adnexal tumours of the skin confirms the sweat gland origin of them. In our experience, however, no conclusions can be drawn in CEA- and AEA-negative cases regarding the origin of the tumour.

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Immunity in herpes gestationis: inhibition of mixed lymphocyte culture by patients' sera

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SUMMARY

The families of four patients with herpes gestationis (HG) (pemphigoid gestationis) and five patients with polymorphic eruption of pregnancy (PEP) were HLA typed. Anti-HLA-D antibodies in the maternal sera were sought using mixed lymphocyte culture (MLC) inhibition test. Two of the four patients with HG had Dw3, one of which was combined with Dw4. One of the fathers had Dw2. The sera of the four patients with HG strongly inhibited (48–100%) the MLC reaction of maternal cells against cells of the father or the child. This kind of inhibition could not be shown in the patients with PEP. We conclude that patients with HG often seem to have MLC inhibiting factors which obviously are antibodies directed against HLA-D region determinants. Their pathogenic role is still obscure.

Herpes gestationis (HG) (also called pemphigoid gestationis) is one of the many disorders associated with HLA antigens. It is weakly associated with HLA-B8, more strongly with HLA-DR3 and very strongly with the combination HLA-DR3/DR4 (Ferrando *et al.*, 1981; Shornick, Stastny & Gilliam, 1981; Holmes *et al.*, 1983).

Some patients with HG seem to have cytotoxic anti-HLA antibodies. We reported one patient (Reunala *et al.*, 1977) and Eberst *et al.* (1981) reported two patients with high titres of cytotoxic anti-HLA antibodies. Stastny *et al.* (1983) searched for antibodies against not only HLA-A, B, and C specificities but also against DR specificities. They found cytotoxic antibodies in as many as 85% of twenty-six patients. Holmes *et al.* (1983) found anti-HLA antibodies in 50% of twenty multigravida patients compared with the surprisingly low figure of 22% in multigravida controls.

In mixed lymphocyte culture (MLC) the reaction starts when the responder cell has recognized a foreign HLA-D antigen on the target cell (a cellular recognition corresponding to the 'antibody + complement' dependent DR recognition). This reaction can be inhibited by

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anti-HLA-D antibodies which block the target D antigen. This is the basis of the MLC inhibition technique, which is a sensitive method for detecting anti-HLA-D antibodies (van Rood *et al.*, 1976). Complement is not needed in this reaction and therefore this technique also reveals non-cytotoxic, i.e. non-complement-binding antibodies.

We HLA-analysed the families of four patients with HG and five patients with polymorphic eruption of pregnancy (PEP) (Jurecka *et al.*, 1983) or 'pruritic urticarial papules and plaques of pregnancy', as it also has been called (Lawley *et al.*, 1979). The MLC inhibition method was used to search for anti-HLA-D antibodies.

METHODS

Patients

The four patients with HG had bullous lesions and deposits of the third component of complement (C3) at the basement membrane zone of both lesional and clinically uninvolved skin. Two patients (A and B) had had two normal pregnancies and HG appeared during the third pregnancy. In the third patient (C) the first pregnancy ended with the birth of a severely malformed child who died soon after delivery. During her second pregnancy the patient had a rash resembling erythema multiforme and during her third pregnancy she had typical HG. The fourth patient (D) developed severe HG during her first pregnancy as described earlier (Reunala *et al.*, 1977). Five years later she became pregnant again, and developed pruritus and C3 deposits in the skin but no rash. The pregnancy ended with an early spontaneous abortion (week 16), after which pruritus disappeared. The time lag between the last delivery or abortion and the search for anti-HLA-D antibodies in the four patients was 4, 1, 6 and 2 years, respectively.

The five patients with PEP had a non-bullous skin eruption with no C3 deposits in uninvolved skin. One of these patients (E) developed the rash during her first, otherwise normal pregnancy. One patient (F) developed the rash during her first pregnancy which ended with the birth of healthy twins. One patient (G) developed the rash during her second pregnancy. She had had a similar rash during the first pregnancy. The fourth patient (H) had previously experienced a spontaneous abortion and the rash appeared during her second pregnancy, which ended with the birth of healthy twins. The fifth patient (I) first had one normal pregnancy with a healthy child. During her second pregnancy she had a pruritic rash, the delivery was normal but the child died within 2 hours of birth for no apparent reason. During her third pregnancy which ended with the birth of a healthy child, she had an eruption resembling erythema multiforme. The time lag between the last delivery and the drawing of the blood sample for the search for anti-HLA-D antibodies in these five patients was 6, 4 and 5 years, 6 months and 6 years, respectively.

HLA typing

HLA-A, B and C antigens were determined with a standard two-stage microlymphocytotoxicity method (Turunen *et al.*, 1979). HLA-D typing was done using local homozygous typing cells for Dw1, w2, w3 and w4 specificities as described earlier (Ilonen *et al.*, 1981).

MLC inhibition tests

Mononuclear cells were isolated from heparinized peripheral blood with Lymphoprep® gradient centrifugation. The washed cells were suspended in a RPMI 1640 medium containing 10% pooled human male serum. Mixed lymphocyte responses between patients and either father or child were performed with 5×10^4 responding cells and an equal number of stimulating irradiated cells. The effect of maternal sera was assayed in microtitre plate triplicate cultures either with 0·05 ml of pooled serum or maternal serum diluted 1:2 with the serum pool. The total volume of cultures was 0·2 ml. They were incubated for 5 days in humidified 5% CO_2 atmosphere with [^{3}H]thymidine present for the last 24 hours. Incorporated radioactivity was measured in a liquid scintillation counter and median c.p.m. values of triplicate cultures were used for calculation of responses. Specific c.p.m. values were calculated by subtracting responses to autologous irradiated cells. Inhibition percentages of maternal sera were calculated with the formula: 100 × (c.p.m. in serum pool − cpm in maternal serum)/c.p.m. in serum pool. To characterize further the inhibition obtained with the maternal sera, these were tested against a panel of two HLA-Dw2 positive (both homozygous) and two Dw2 negative subjects as stimulating cells in MLC.

RESULTS

The HLA genotypes in families with HG are shown in Table 1. Two of the four patients had HLA-Dw3, in one patient in combination with Dw4. One of the fathers had Dw2. In family A the mother and the father were HLA-identical with the exception of one D antigen, which was Dw1 in the mother and 'blank' in the father.

The HLA genotypes of the five families with PEP are shown in Table 2. Two of the patients had Dw3.

Table 3 shows the results of MLC inhibition tests in families with HG and PEP. All sera from patients with HG were inhibitory in MLC cultures. In three of the four patients the inhibition was stronger when cells with paternal antigens acted as targets for the responding maternal cells than in a reversed experimental design. In the families with PEP, MLC inhibition was studied in four cases. In two families the mother did not respond to the cells of her husband. In the other two families only slight or no inhibition was seen.

Table 4 shows the MLC inhibition obtained with maternal sera in MLC cultures where maternal cells of patients with HG or PEP were stimulated with Dw2 positive or negative cells from individuals unrelated to the patients. Two of the sera of patients with HG strongly inhibited the response induced by Dw2 cells. In the case of serum D the inhibition was highly specific, but serum B also inhibited almost as strongly responses induced by other cells. Serum

TABLE 1. HLA genotypes of families with herpes gestationis

Family	Mother	Father	Children	
A	A2;B15,w6;Cw3;Dw1 A3;B7,w6;Cw7;Dw2	A2;B15,w6;Cw3;D- A3;B7,w6;Cw7;Dw1	m	A2;B15,w6;Cw3;D- A3;B7,w6;Cw7;Dw2
B	A2;B40,w6;C-;D- A3;B12,w4;C-;D-	A2;B15,w6;Cw3;Dw4 Aw19;B7,w6;Cw7;Dw2	m	Aw19;B7,w6;Cw7;Dw2 A2;B40,w6;C-;D-
C	A1;B8,w6;C-;Dw3 A3;Bw35,w6;Cw4;Dw1	A3;B15,w6;C-;D- A10;B15,w6;Cw4;Dw1	m	A10;B15,w6;Cw4;Dw1 A3;Bw35,w6;Cw4;Dw1
			m	A3;B15,w6;C-;D- A1;B8,w6;C-;Dw3
D	A3;B15,w6;C-;Dw4 A3;Bw35,w6;Cw3;Dw3	A1;B8,w6;C-;Dw3 A2;B40,w6;Cw2;D-	f	A1;B8,w6;C-;Dw3 A3;B15,w6;C-Dw4

TABLE 2. HLA genotypes in families with polymorphic eruption of pregnancy

Family	Mother	Father	Children
E	A1;B8,w6;C-;Dw3 A3;B7,w6;C-;Dw6	A2;B8,w6;C-;Dw3 A3;B27,w4;Cw1;D-	m A2;B8,w6;C-;Dw3 A3;B7,w6;C-;Dw6
F	A2;B27,w4;Cw1;Dw1 A9;Bw16,w6;C-;D-	A3;Bw35;w6;Cw4;Dw1 A3;Bw35,w6;Cw4;Dw1	m A3;Bw35,w6;Cw4;Dw1 A2;B27,w4;Cw1;Dw1
			f A3;Bw35,w6;Cw4;Dw1 A9;Bw16,w6;C-;D-
G	A28;B12,w4;C-D- Aw19;B5,w4;C-;D-	A2;B27,w4;Cw2;D- A10;B8,w6;C-;Dw3	m A2;B27,w4;Cw2;D- A28;B12,w4;C-;D-
H	A1;B8,w6;C-;Dw3 A9;B27,w4;Cw1;D-	A2;B7,w6;Cw7;Dw2 A9;Bw16,w6;C-;D-	f A2;B7,w6;Cw7;Dw2 A9;B27,w4;Cw1;D-
			f A2;B7,w6;Cw7;Dw2 A9;B27,w4;Cw1;D-
I	A2;B18,w6;C-;D- A3;B7,w6;C-;Dw2	A2;B13,w4;Cw6;D- A2;B40,w6;C-;D-	f A2;B40,w6;C-;D- A2;B18,w6;C-;D-
			m A2;B13,w4;Cw6;D- A3;B7,w6;C-;Dw2

TABLE 3. MLC responses (specific c.p.m.) in families with herpes gestationis and polymorphic eruption of pregnancy and the effect of maternal serum on the responses

	Reaction of maternal cells against irradiated paternal cells			Reaction of paternal cells against irradiated maternal cells		
Family	Serum pool	Maternal serum	Inhibition (%)	Serum pool	Maternal serum	Inhibition (%)
Herpes gestationis						
A	17,617	8977	48	7798	3098	60
B	12,644	1054	92	7996	2234	72
C	22,872	8160	64	6214	4558	27
D	2530	0	100	4452	653	85
Polymorphic eruption of pregnancy						
E	6524	6901	0	6523	6019	8
F	382	1360	0	8053	6317	22
G	9414	8483	10	1066	2232	0
I	0	0	—	12,587	5310	58

TABLE 4. The effect of maternal sera (inhibition %) on the MLC response of maternal cells against unrelated Dw2 positive or negative stimulating cells in patients with herpes gestationis and polymorphic eruption of pregnancy

	HLA-D type of the stimulating cell			
Patient	Dw5/8	Dw1/–	Dw2/2	Dw2/2
Herpes gestationis	Inhibition (%)			
A	5	35	19	9
B	77	71	95	80
C	61	26	27	26
D	−9	9	82	85
Polymorphic eruption of pregnancy	Inhibition (%)			
E	19	21	39	23
F	−1	−4	47	13
G	32	40	44	28
H	27	6	19	9
I	86	94	68	67

C strongly inhibited the response induced by a Dw2 negative cell. Serum A did not inhibit any of the responses to the same extent as with paternal cells. The sera of patients with PEP were also tested. One of these (I) inhibited all responses, but especially the responses induced by two HLA-Dw2 negative cells. Other sera did not show inhibitions over 50%.

DISCUSSION

The results of the HLA analyses of the four patients with HG agreed with the earlier reports of increased frequency of HLA-DR3 and DR3/DR4 combination in HG.

The high titres of cytotoxic anti-HLA-A, B and C antibodies found occasionally (Reunala *et al.*, 1977; Eberst *et al.*, 1981) became more interesting when Stastny *et al.* (1983) showed that most patients with HG have cytotoxic anti-HLA antibodies when also anti-D/DR antibodies are looked for. The MLC inhibition test is a sensitive method for demonstrating anti-HLA-D antibodies, which cannot be detected with ordinary cytotoxicity tests. With this test we showed that all our four patients with HG had in their sera a potent MLC inhibiting factor, which is most likely an anti-HLA-D antibody. These factors could be found although in most cases the study was done years after the last delivery. During pregnancy the prevalence and strength of these factors may be even greater. Similar MLC-inhibiting antibodies also occur during the last trimester of normal pregnancy (Herva & Tiilikainen, 1977), but in women with few pregnancies they occur in significant potency after delivery in only about 20% of subjects studied (Herva & Jouppila, 1977). The actual prevalence of antibodies directed against the HLA region should be confirmed in a larger group of patients with HG, using methods like the MLC inhibition test which detect non-cytotoxic antibodies also.

The panel of HLA-Dw2 positive and negative subjects was selected for testing because the frequency of DR2 has been reported to be increased among the partners of HG patients (Shornick *et al.*, 1982). Only one of the fathers in our material had Dw2, a common antigen in the Finnish control population. In this case the maternal serum (B) seemed to have specificity for Dw2. However, another serum (D) was also highly specific for Dw2, although Dw2 antigen was not found in the cells of the child (Dw3/Dw4) whose cells were also strongly inhibited as stimulators in MLC. Most probably the specificity is not directed against Dw2, but against some other determinant coded by a gene in the HLA region and possessing characteristics similar to HLA-D antigens. The pattern of inhibition in Table 4 is completely heterogeneous and thus among the specificities of the sera there is no mutual component.

The role of anti-HLA antibodies in the pathogenesis of HG is totally speculative. HG is not likely to be a pure hypersensitivity reaction of the mother to the foreign fetal HLA antigens, because only a few of the patients have cytotoxic anti-HLA antibodies strong enough to explain a severe disease. Anti-HLA antibodies could, however, interfere in some other way with the complex immunological relations between the mother and the fetus and thus actively participate in the pathogenesis of HG. On the other hand it has also been suggested that the increased tendency to produce anti-HLA antibodies is only a result of the high general immune responsiveness of patients with HG (Holmes *et al.*, 1983).

We conclude that cytotoxic anti-HLA-A, B and C antibodies are found only occasionally in patients with HG, but that anti-HLA-D/DR antibodies occur in most patients with HG. The MLC inhibition test is a sensitive method for detecting non-cytotoxic anti-HLA-D antibodies. It is not known whether these cytotoxic or non-cytotoxic anti-HLA antibodies play any role in the pathogenesis of HG.

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The effect of serum calcium levels on the rate of epidermal renewal in the rat

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SUMMARY

The growth and differentiation of epidermal cells *in vitro* show a marked dependence on the calcium concentration of the medium. In this study the effect of experimentally produced hyper-and hypocalcaemia on the rat epidermis *in vivo* has been investigated. Hypercalcaemia, induced by injections of calcium chloride, produced a decrease in epidermal labelling index and some epidermal thinning. On the other hand hypocalcaemia, induced by calcitonin, failed to lead to changes in these measurements. The diurnal variation in epidermal labelling index and serum calcium levels was also measured. Whilst the labelling index decreased considerably over the period 09.00 hours to 18.00 hours, no significant changes were observed in serum calcium. These results suggest that while, under certain circumstances *in vivo*, the epidermal cell shows the same sensitivity to calcium as it does *in vitro*, calcium is not a major regulator of epidermopoiesis.

There is now considerable evidence that calcium and its intracellular receptor calmodulin participate in the regulation of a wide range of cellular processes (Means & Dedman, 1980; Stoclet, 1981; Denton & McCormack, 1981). These include not only physiological events, such as muscle contraction (Fabiato & Fabiato, 1979) but also cellular and biochemical events such as endocrine secretion (Rasmussen & Goodman, 1977) and cyclic nucleotide metabolism (Wolff & Brostrom, 1979). Epidermal cell activities are also apparently regulated by calcium ions. Experiments with mouse (Hennings *et al.*, 1980), rat (Brysk & Snider, 1982) and human (Dykes, Jenner & Marks, 1982; Boyce & Ham, 1983) epidermal cells *in vitro* have all demonstrated that their growth and differentiation may be modulated by this divalent cation.

At low calcium levels (0·05–0·2 mM), epidermal cells show increased plating efficiency and increased growth rate. In addition, cultures tend to exist as monolayers and ultrastructurally show widened intercellular spaces. In high calcium concentrations (1·2–2·0 mM) the rate of cell

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growth is reduced. Colonies are multilayered and show increases in markers of differentiation such as transglutaminase activity (Hennings, Steinert & Buxman, 1981). The existence of such a simple and dramatic means of controlling the growth and differentiation of epidermal cells *in vitro*, raises the question as to whether such a mechanism might operate *in vivo*.

In order to define the relationship between extracellular calcium concentration and epidermopoiesis *in vivo*, the effect of experimentally produced hypo- and hypercalcaemic states on the rat epidermis has been investigated in this study. The epidermal labelling index and mean epidermal thickness have been used as measures of the rate of epidermal renewal.

METHOD

Animals

Male Sprague Dawley rats weighing 125–175 g were used in this study. The animals were housed and maintained under standard laboratory conditions.

Determination of epidermal labelling index

This was determined *in vitro*. Animals were anaesthetized with diethyl ether and killed by cervical dislocation. After shaving with electric clippers a Silver's knife biopsy of the epidermis and superficial dermis was taken. This was cut into small fragments (2 × 5 mm) and incubated in Eagle's minimal essential medium containing ^{3}H-labelled thymidine for 4 hours (Francis & Marks, 1977). After incubation, specimens were fixed in phosphate-buffered formalin and processed by routine methods for autoradiography. The percentage of labelled basal and suprabasal cells of the total number of basal epidermal cells was then determined microscopically.

Determination of mean epidermal thickness

Full thickness skin specimens were taken at the same time as the Silver's knife biopsies. These were fixed in phosphate-buffered formalin and processed by routine histological methods. The mean epidermal thickness was measured using the Quantimet 720 image analysis system (Gaskell & Pearse, 1981).

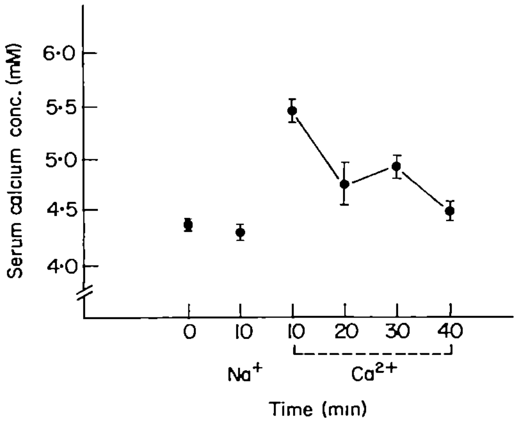

FIGURE 1. Effect of a single intraperitoneal injecion of 1 ml 62·5 mM calcium chloride on serum calcium concentration. Results shown are for no treatment (time = 0 min), 10 min after injection of 1 ml 125 mM sodium chloride (time = 10 min, Na^{+}) and 10, 20, 30 and 40 min after injection of 1 ml 62·5 mM calcium chloride. Each point represents the mean ± s.e.m. for four rats.

Induction of hypo- and hypercalcaemia

Hypercalcaemia was induced by intraperitoneal injection of 1 ml 62·5 mM calcium chloride (Perris, Whitfield & Rixon, 1967). Control animals received 1 ml 125 mM sodium chloride (chosen to give the animals an equivalent amount of chloride ions).

A single intraperitoneal injection of calcium chloride produced only transient hypercalcaemia (Fig. 1). At 10 minutes after injection serum levels were increased by approximately 25%. However, by 40 minutes values had returned to the normal range. Therefore, to maintain hypercalcaemia, rats were injected at hourly intervals for a period of 8 hours. Animals were killed and skin samples taken either immediately (8 hours) or the next morning (24 hours).

Hypocalcaemia was induced by subcutaneous or intraperitoneal injection of porcine calcitonin in gelatin (Calcitare®, Armour Pharmaceutical Co., Ltd). Control animals received either gelatin or saline alone.

Serum calcium measurements

At the time of death, blood samples were taken either by heart puncture or from the inferior vena cava. These were allowed to clot at room temperature, ringed and incubated at 37°C for 1 to 2 hours. Serum was removed, centrifuged (2000 ***g*** × 15 min) to remove contaminating red blood cells, and stored at −20°C. When required, samples were thawed, diluted in de-ionized water and the calcium concentrations measured by atomic emission spectroscopy.

RESULTS

Results of the labelling index and mean epidermal thickness measurements in hypercalcaemic rats are presented in Table 1. There was no significant difference at 8 hours between calcium chloride and control rats. On the other hand at 24 hours the labelling index was significantly lower in the calcium chloride injected animals ($P < 0{\cdot}001$) (Student's *t*-test). Fewer injections of calcium chloride were not effective in reducing the labelling index. In a subsequent experiment (see Fig. 2), two, four and eight injections of calcium chloride were compared with eight injections of sodium chloride. At 24 hours only those animals receiving eight injections showed a decrease in labelling index.

TABLE 1. Effect of hypercalcaemia on rat epidermis

Time (hours)	Treatment*	*n*	Labelling index (%) (Mean ± s.e.m.)	Mean epidermal thickness (μm) (Mean ± s.e.m.)
8	NaCl (125 mM)	6	2·53 ± 0·43	22·95 ± 0·69
8	$CaCl_2$ (62·5 mM)	6	1·68 ± 0·16	24·25 ± 1·18
24	NaCl (125 mM)	6	8·62 ± 0·34	21·32 ± 1·28
24	$CaCl_2$ (62·5 mM)	6	2·28 ± 0·44**	19·12 ± 0·68

* Given as 8 × 1 ml injections intraperitoneally at hourly intervals.

** Significantly different from control, $P < 0{\cdot}001$ Student's *t*-test.

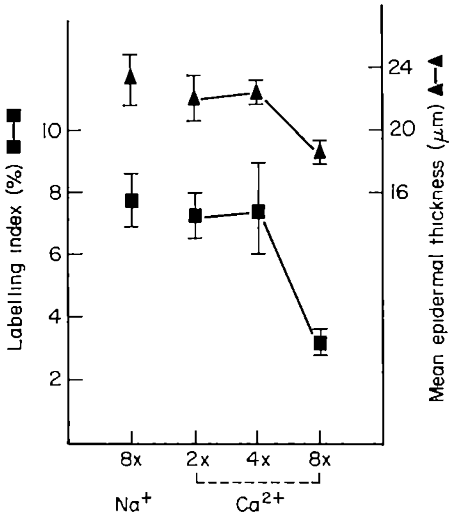

FIGURE 2. Effect of increasing numbers of injections of calcium chloride on epidermal labelling index and mean epidermal thickness. Results shown are for groups of animals receiving either eight injections of 1 ml 125 mM NaCl or two, four and eight injections 1 ml 62·5 mM $CaCl_2$. The labelling index (LI) and mean epidermal thickness (MET) were determined at 24 hours. Each point represents the mean ± s.e.m. for four rats.

Analysis of variance indicated that the differences in labelling index were significant ($P = 0{\cdot}019$), whereas the differences in mean epidermal thickness were borderline ($P = 0{\cdot}055$).

The results presented in Table 1 also suggested diurnal variation of the labelling index. The control values at 8 hours (18.00 hours) were considerably lower than those at 24 hours (9.00 hours). This was confirmed in a subsequent experiment where untreated rats were killed at different times during the day and samples taken for determination of epidermal labelling index

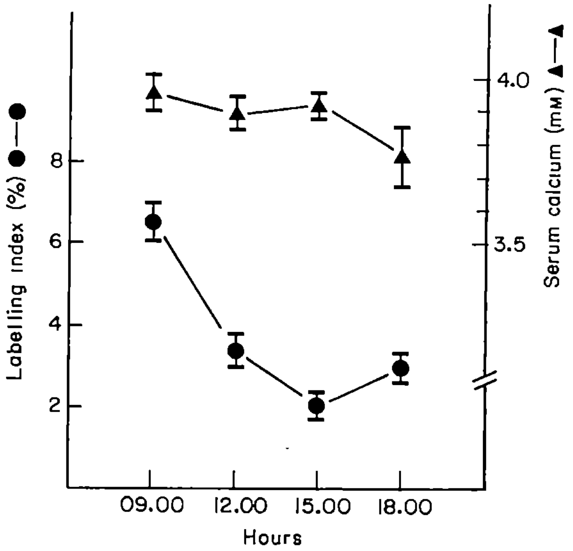

FIGURE 3. Diurnal variation in epidermal labelling index and serum calcium level in normal rats. Each point represents the mean ± s.e.m. for five rats.

TABLE 2. Effect of calcitonin-induced hypocalcaemia on rat epidermis

	n	Labelling index (%) (Mean ± s.e.m.)	Mean epidermal thickness (μm) (Mean ± s.e.m.)
Calcitonin (1 14 units)	6	6 42 ± 0·51	21·13 ± 0·69
Control	6	6 01 ± 0 65	20 63 ± 0·82

and serum calcium levels. The results are presented in Fig. 3. As can be seen the values for labelling index at 09.00 hours are much higher than for samples taken at 12.00, 15.00 and 18.00 hours. The levels of serum calcium showed little variation over this period. One-way analysis of variance of the serum calcium values indicated no significant differences between the time points chosen ($P = 0{\cdot}110$).

A single injection of 1 unit calcitonin produced prolonged hypocalcaemia with serum levels approximately 10% below normal (data not shown). These changes did not lead to differences in labelling index or epidermal thickness at 24 hours (see Table 2).

DISCUSSION

The circulating, and presumably extracellular, tissue levels of calcium are normally controlled within fairly strict limits by a combination of calcitonin, parathyroid hormone and vitamin D. Clinically, changes in the skin are often seen in hypocalcaemia due to hypoparathyroidism. Loss of hair, nail abnormalities and roughness and 'dryness' of the skin have been reported in approximately one-third to one-quarter of patients with both post-thyroidectomy and idiopathic hypothyroidism (Braverman, 1981). There are also reports of impetigo herpetiformis and relapses in psoriatic patients being associated with hypocalcaemia. In addition, experimentally induced changes in serum calcium in the rat have been shown to affect cell turnover in organs such as the thymus and bone marrow (Perris *et al.*, 1967). DNA synthesis in healing skin wounds in rabbits is also reported to be stimulated in calcitonin-injected animals (Lupulescu, 1975).

The results presented here clearly show that experimentally produced hypercalcaemia inhibited epidermal cell division as measured by the thymidine labelling index method. Although thymidine labelling techniques have many pitfalls (Maurer, 1981), the fact that epidermal thinning was frequently seen in this type of experiment (see Fig. 2) indicated that inhibition of cell division rather than some other effect was occurring. As such the hypercalcaemia experiments show a parallel to the *in vitro* experiments previously reported, i.e. high calcium concentrations in the medium inhibited cell division. In contrast, experimentally induced hypocalcaemia had no effect on epidermal labelling index. *In vitro* observations would have predicted an increase in this value.

No attempt has been made in this study to measure the extravascular levels of calcium in the skin. Technically it would be extremely difficult and the contribution of blood vessels, cells and other structures in the skin would be difficult to assess. However, it is unlikely that the blood vessel walls themselves represent a barrier to the diffusion of small ions such as calcium, and it seems probable that the extravascular calcium levels follow closely the circulating levels. The lack of response of the epidermis to hypocalcaemia may indicate that a stimulating effect of hypocalcaemia operates only under certain circumstances *in vivo*. Further evidence to support this conclusion comes from the studies on diurnal variation (Fig. 3). Although the data do not cover the full 24-hour period, the results do not support the idea of serum calcium being a major regulator of epidermal turnover. Thus, while the serum calcium levels showed only small and statistically insignificant changes, the labelling index fluctuated dramatically.

The mechanism by which hypercalcaemia inhibits cell turnover is a matter for speculation. One possibility is via cytoplasmic calcium binding proteins. Calmodulin has been identified in the epidermis (Murray & Rogers, 1978; Iizuka *et al.*, 1982) as well as other, possibly related vitamin D dependent calcium-binding proteins (Saurat *et al.*, 1981). Another possibility would be alterations in subcellular distribution of calcium, particularly that associated with the plasma membrane. Such changes would lead to alterations in membrane transport and permeability and subsequently cell turnover.

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Pharmacology and Treatment

The treatment of resistant warts with intralesional bleomycin: a controlled clinical trial

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SUMMARY

A controlled double-blind trial was carried out on fifty-nine matched pairs of hand warts in a group of twenty-four patients, with proven resistance to treatment, to study the effectiveness of the intralesional injection of 0·1% solution of bleomycin sulphate in normal saline, compared with a normal saline placebo injected into the paired warts in the same patient. We found that 87·5% of patients showed a more favourable response to bleomycin ($P < 0.001$); 76% of the fifty-nine warts treated with bleomycin were cured by one to three injections of up to 0.2 ml of solution. Subsequently 75% of thirty-two patients with resistant hand warts and 66% of fifteen patients with mosaic plantar warts were cured of all their warts. No patient received more than 4 mg of bleomycin. No toxicity was experienced. Local pain was on the whole well tolerated. This form of treatment for resistant warts is reliable, safe and acceptable to patients.

Seventy to eighty per cent of patients with viral warts can be cured within 3 months by conventional methods of treatment (Bunney, Nolan & Williams, 1976) but there remains a hard core whose warts persist despite prolonged and rigorous attack. Resistance to treatment may prove to be a property of viral type, or the amount of virus present in the warts (Jenson, Sommer & Payling Wright, 1982), but there is growing evidence that the principal factor is the inability of the patient to mount an adequate immunological response (Jablonska, Orth & Lutzner, 1983). The frankly immunodefective or immunosuppressed individual presents a real therapeutic problem.

The intralesional injections of warts with the anti-neoplastic/antibiotic drug bleomycin is a promising new approach in view of its unusual properties of preferential binding in squamous cells, DNA strand scission and restricted toxicity. Bleomycin is already widely used for the treatment of warts in Japan (Sayama & Tagami, 1983), and is licensed for this purpose in

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Canada. There is a growing number of reports of the successful intralesional injection of warts using very small doses of bleomycin, well below the lowest reported toxic dose of 90 mg (Bremner, 1976; Hudson, 1976; Olson, 1977; Abbott, 1978; Shumack & Haddock, 1979; Cordero, Guglielmi & Woscoff, 1980; Bakker, 1982). In no case was the cumulative dose greater than 5 mg and no toxic side-effects were experienced. Four groups reported a cure rate of over 70%. Olson (1977) and Shumack & Haddock (1979) both included a few control cases in their studies who failed to respond to saline injection, but responded subsequently to bleomycin. It was considered that further investigation of this form of treatment for persistent warts was warranted, and permission was obtained from the Committee on Safety of Medicines to carry out a properly controlled trial.

METHODS

Patients

We decided to use patients as their own controls in view of our past experience of the wide variations in response to treatment between patients (Bunney *et al.*, 1976).

Patients were chosen who had had hand warts for more than 9 months, who had been treated by us, without improvement, for at least 3 months and who had one or more pairs of warts matched for type, site and size.

Materials

Bleomycin sulphate solution was prepared in the hospital sterile products unit by dissolving 5 mg of the powder in 5 ml of sterile normal saline. Normal saline was used as the placebo.

For each numbered patient, the pharmacist prepared two 1 ml tuberculin syringes labelled A and B containing, randomly, 0·5 ml of either bleomycin solution or saline. The syringes were prepared freshly on the day of use to avoid the loss of potency caused by storage of bleomycin solution in plastic (Benvenuto *et al.*, 1981).

Randomization lists were held by the pharmacist and statistician only and the code was not broken until completion of the trial.

Trials

On entry, for each trial patient a trial number was allocated. A history relevant to wart resistance was taken; all hand warts present were charted, measured and numbered. Matched pairs of warts were identified. One wart in each pair was injected with solution A, and the other with solution B, 0·1 ml into warts under, and 0·2 ml into warts over, 5 mm in diameter. A 25 Luer needle was used for injections with the bevelled edge upwards. Injection was made directly into the wart tissue. If the wart was large a divided dose was injected into two sites to obtain even distribution of the solution. The injection sites were covered with simple strapping which was removed by the patient the next day.

At 3 weeks warts were assessed 'blind' and independently by two of us. A wart was considered cured only when the skin texture at the site was restored to normal. If assessments differed a joint assessment was agreed. Uncured warts were reinjected with the same solution A or B that they had received previously.

At 6 weeks warts were assessed as at 3 weeks. A decision was then made as to whether A or B was active for that patient. Any remaining trial warts were reinjected with the 'active' solution, or in case of doubt with labelled bleomycin.

At 12 weeks a final assessment of the state of the trial warts was made.

Blood samples were taken at the beginning and end of the trial for a full blood count, E.S.R., anti-DNA binding and wart antibody estimations.

At each visit the health of the patient, and any side-effects or pain experienced were recorded.

RESULTS

Twenty-four patients entered the trial. There were ten males and fourteen females whose ages ranged from 15 to 59 years with a median of 32 years. The duration of warts ranged from 4 months to 28 years with a median of 3 years—four patients had had their warts for more than 10 years. The duration of previous treatments varied from 3 months to 11 years with a median of 12 months. Fifty-nine pairs of matched warts were treated.

Assessment of the outcome at 6 weeks

The assessment of results at 6 weeks was made on the current state of the warts in twenty patients, but on the state of the warts at 3 weeks in four patients, three of whom failed to attend at 6 weeks but did so subsequently and one who had insisted that all her warts should be reinjected with what she considered to be the active solution at 3 weeks. The outcome is shown in Table 1. Twenty-one patients (87·5%) showed a more favourable reaction to bleomycin than saline. In one patient with a single pair of warts, both were cured, so spontaneous resolution could not be excluded. In one patient both bleomycin and saline appeared equally effective, and in one, neither caused much change. The outcome in the fifty-nine matched pairs of warts is shown in Table 2. Amongst the bleomycin-treated warts thirty-four (58%) were cured, thirteen were nearly cured, nine were smaller and only three (5%) remained unchanged. Amongst the

TABLE 1. Outcome at 6 weeks of twenty-four patients

Outcome	No. of patients	
Bleomycin more favourable	21 (87 5%)	
Both curative (one pair warts)	1	$P < 0.001$
Equally responsive	1	Sign Test
Equally ineffective	1	
Total	24	

TABLE 2. Outcome at 6 weeks: fifty-nine pairs of warts (treated randomly and blind)

	No. of warts	
Outcome	Bleomycin-treated	Saline-treated
Cured	34 (58%)	6 (10%)
Traces	13	7
Smaller	9	2
Unchanged	3 (5%)	44 (75%)
Total	59	59

saline-treated warts only six (10%) were cured, seven nearly cured, two smaller, whilst 44 (75%) remained unchanged.

These results show that bleomycin is effective in curing warts to a highly significant degree ($P < 0{\cdot}001$, Sign Test).

Reinforcement of results

At 6 weeks, three patients defaulted, three withdrew from further participation, one on account of apparent failure, one because of distance, and one from dislike of injections. Three patients were cured of all their warts, including the patient who had received the 'active' solution in all her warts at 3 weeks. Fifteen patients therefore received a further injection into all remaining trial warts with the 'active' solution, or labelled bleomycin in the single case where it was not clear whether A or B was active. After breaking the code at the conclusion of the trial it was found that the conjectured active agent was bleomycin in all cases.

At 12 weeks in these fifteen patients, six more of the thirteen warts previously treated with bleomycin were cured, as were fifteen of the thirty-six previously treated with saline. No wart remained unchanged (Table 3).

TABLE 3. Outcome at 12 weeks: fifteen patients, one injection bleomycin (fourteen blind, one labelled)

	No. of warts	
Outcome	Previous bleomycin	Previous saline
Cured	6 (46%)	15 (41%)
Affected	7	21
Unchanged	0	0
Total	13	36

Five of six bleomycin-treated warts in the three patients who had defaulted at 6 weeks were also found to have been cured. The sixth was cured within 3 weeks by a third injection of bleomycin. The outcome in all fifty-nine warts treated with bleomycin was therefore known: forty-five (76%) were cured. The highest cumulative dose given to any patient was 1·2 mg.

Extension of the trial

Whilst the ability of bleomycin to cure individual warts is impressive, a treatment cannot be considered successful unless the patient can be completely cured of all warts. We were able to continue treating sixteen of the trial patients who still had warts, many of which had not been treated during the trial. Three patients had more than twenty warts: one of these was cured, one with an acute lymphoma continued to develop new warts and the third remains under treatment. Five patients had ten to twenty warts: three were cured, one underwent an apparent spontaneous resolution and the fifth remains under treatment. All six of those with five to ten warts and both of those with less than five were cured. In all, twelve (75%) of the patients were cured (Table 4). Three other patients had already been cured of all their warts in the course of the trial. They had two, four and six warts respectively. The highest cumulative dose of bleomycin for any patient was 4 mg.

TABLE 4. Follow-up treatment with bleomycin of sixteen patients

No. warts present	No. of patients	No. patients cured
> 20	3	1
10–20	5	3
5–10	6	6
< 5	2	2
Total	16	12 (75%)

Parallel study

To obtain additional information we treated a further thirty-two patients with highly resistant warts whom we had been unable to include in the trial as they had no matching pairs of warts. There were six males and twenty-six females whose ages ranged from 14 to 76 years (median 32 years); the duration of warts was 18 months to 29 years (median 3 years). They had received between three and 100 previous treatments (median fourteen treatments). Sixteen had hand warts including peri- and subungual warts, and fifteen had mosaic plantar warts. Twelve (75%) of those with hand warts and ten (66%) of those with mosaic plantar warts were cured (Table 5). The highest cumulative dose of bleomycin in this group was 2·5 mg.

Follow-up

All fifty-six patients have been followed up for more than 6 months. Only two patients had recurrences of any bleomycin-treated warts. One was the patient with an active lymphoma receiving radiotherapy. The other was a patient with a raised anti-DNA binding level and Raynaud's phenomenon. Her warts responded to a further single dose of bleomycin as did those of three patients who each developed a new small wart at a distant site.

Side-effects

None of the fifty-six patients treated experienced any toxic side-effects and no changes in blood counts were observed. Patients found injections with bleomycin slightly painful but only one

TABLE 5. Parallel study: thirty-two patients treated with bleomycin

	No. of patients	
Outcome	Hand warts	Mosaic plantar warts
Cured	12 (75%)	10 (66%)
Traces	1	2
Withdrawn	1	3
Defaulted	1	0
Continues	1	0
Total	16	15

withdrew on this account. Most patients experienced some pain on the day following injection, this was severest in those with plantar and periungual warts. It rarely lasted longer than 48 hours and only two withdrew because of it. Most patients preferred treatment with bleomycin to cryotherapy.

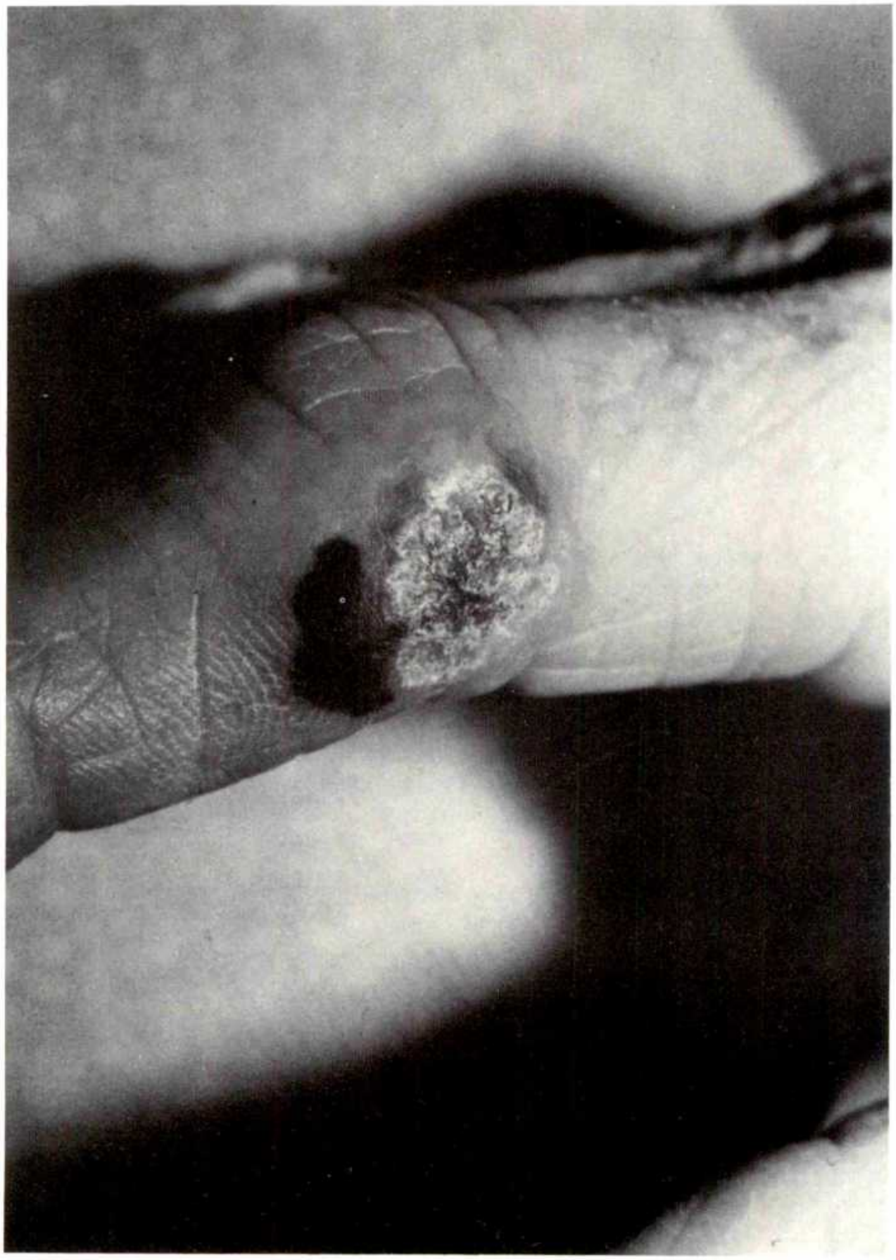

FIGURE 1. Appearance of wart 1 week after successful injection with bleomycin showing black eschar.

Clinical response to intralesional bleomycin

Warts injected with bleomycin oozed more than usual when the needle was withdrawn but haemostasis was no problem. The day following treatment the injected wart became red and painful, rapidly turning black. By 1 week a blue-black eschar was formed (Fig. 1). This was slow to separate but in most cases had done so within 3 weeks, resulting in complete healing without scarring (Fig. 2). In some cases the eschar was not detached at 3 weeks, but could be scraped away leaving a pitted area which had healed completely by the next visit. If infiltration had been inadequate only part of the wart turned black and separated leaving residual wart tissue.

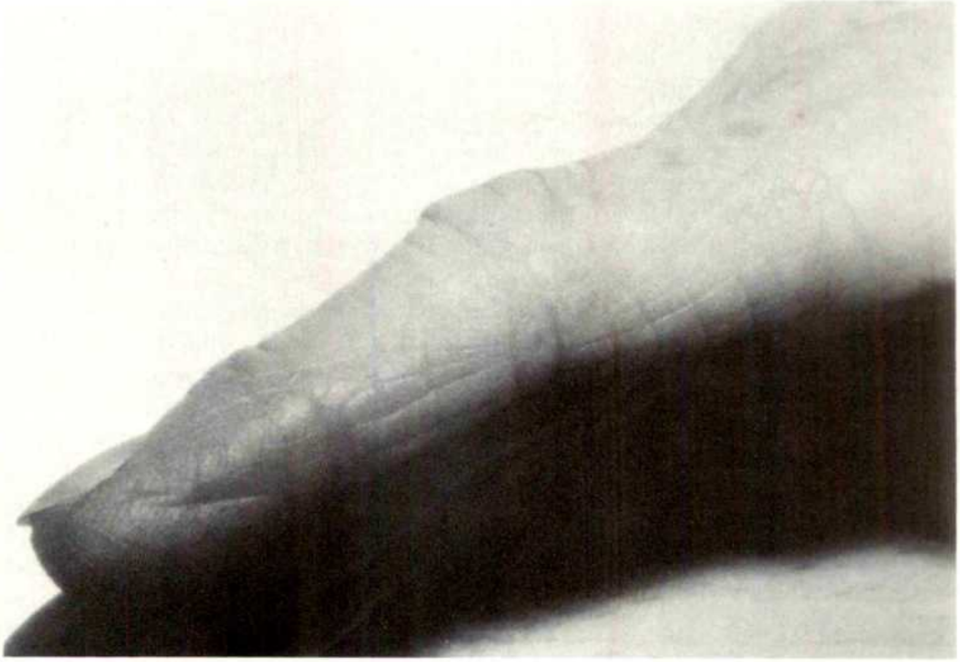

FIGURE 2. Complete cure of wart shown in Fig. 1.

DISCUSSION

In view of the very distinctive changes following bleomycin injection which were never seen in saline-injected warts, every effort was made to make a blind assessment, so patients were not examined until 3 weeks after injection. Nor were they questioned about the changes they had noticed until the assessment had been made.

The interval of 3 weeks between treatments was chosen in the light of our previous experience that the cure rate of warts was halved when the interval between freezing treatments was greater than 3 weeks (Bunney *et al.*, 1976). In the event, however, it would have been better to use a 4-week interval in view of the slowness of separation of the eschar leaving numbers of warts incompletely healed at 6 weeks, which had disappeared without further treatment by 12 weeks. Assessment at 8 weeks might well have shown an even higher level of effectiveness of bleomycin.

Adequate infiltration, indicated by blanching of the wart, was found to be crucial to a successful outcome. To this end anaesthesia of the area with 2% lignocaine prior to the injection of peri- and subungual warts can be helpful, and paring of keratotic warts is necessary. Previously injected warts were easier to infiltrate on subsequent occasions. It was difficult to prevent leakage of the solution from between the filiform projections of some exophytic warts. Professor Willoughby of Halifax, Nova Scotia, who was one of the pioneers of this form of treatment in Canada and has now treated several thousand patients successfully, recommends the application of pressure to the surface of the wart with a gloved finger and injecting as near the base of the wart as possible. A dermojet was used by Olson (1977) and by Hudson (1976) in their studies, but Munkvad *et al.* (1983) did not find this effective and suggest that the scatter of the solution results in too small a dose being delivered within each area. We found that our results were better using a 25 rather than 27 Luer needle.

Willoughby (1978) suggests that the acute pain reported by some is due to the injection being made too deeply causing a sublesional haematoma. Only two of our patients, both with plantar warts, withdrew because of pain, and two more complained of quite severe pain following injection of periungual warts, but were willing to continue treatment as they saw it to be effective. The length of time required to cure a patient of warts depends not only on the skill of the operator, but the number of warts present, and how many injections the patient can tolerate in one session. The confidence with which treatment is given, is based upon an understanding of the properties and mode of action of bleomycin.

The isolation by Umezawa of the bleomycins from *Streptomyces verticillus*, a Japanese soil fungus, in 1962 has been fully described (Umezawa *et al.*, 1966). Pharmaceutical bleomycin sulphate contains a number of natural bleomycins, standardized to the Japanese specification. It is stable in powder form and is usually packed in 15 mg vials. It is fully soluble in water and normal saline. Whilst solutions remain stable for up to 60 days if stored in glass at 4°C, it has been suggested that sterility may not be maintained for that length of time. However, samples taken from our own multi-dose ampoules at 4 and 8 weeks have proved sterile.

Bleomycin has both antibiotic and anti-neoplastic properties depending on its ability to cause DNA strand scission, especially in dividing cells entering the mitotic phase (Saunders & Schultz, 1972). Umezawa (1974) demonstrated that bleomycin inactivates SV40 by scission of both strands of DNA so it is possible that in addition to its cytotoxic effect on the cells of the wart, it may also act upon the double-stranded DNA of the HPV. Bleomycin given systemically is rapidly dispersed throughout the body tissues, and has a half life of 2 hours and all has been excreted in the urine within 72 hours (Bennet & Reich, 1979). Bleomycin is inactivated by an intracellular aminopeptidase enzyme designated bleomycin hydrolase (Umezawa, 1974). The bone marrow, red cells, liver and spleen are rich in this enzyme so haemopoetic and hepatic or renal toxicity do not occur (Ohnuma *et al.*, 1974). The immunological system is unaffected (Yagoda *et al.*, 1974). On the other hand the skin and lungs contain little of the hydrolase and signs of toxicity appear first in the skin in the form of erythema, pruritus, hyperpigmentation and hair loss. Cohen *et al.* (1973) and Mountz *et al.* (1983) have described scleroderma-like changes following high dosage. The most serious complication is interstitial fibrosis of the lungs. All the toxic side-effects are dose-related, those in the skin occurring after the administration of 150 mg, and those in the lungs when over 450 mg have been given. Furthermore, because of the rapid elimination of the drug the toxicity can be diminished by spacing of administration and the toxic effects are in general reversible. At the level of dosage used in the treatment of warts toxicity is not anticipated.

The paucity of bleomycin hydrolase in the skin results in the firm and localized binding of the

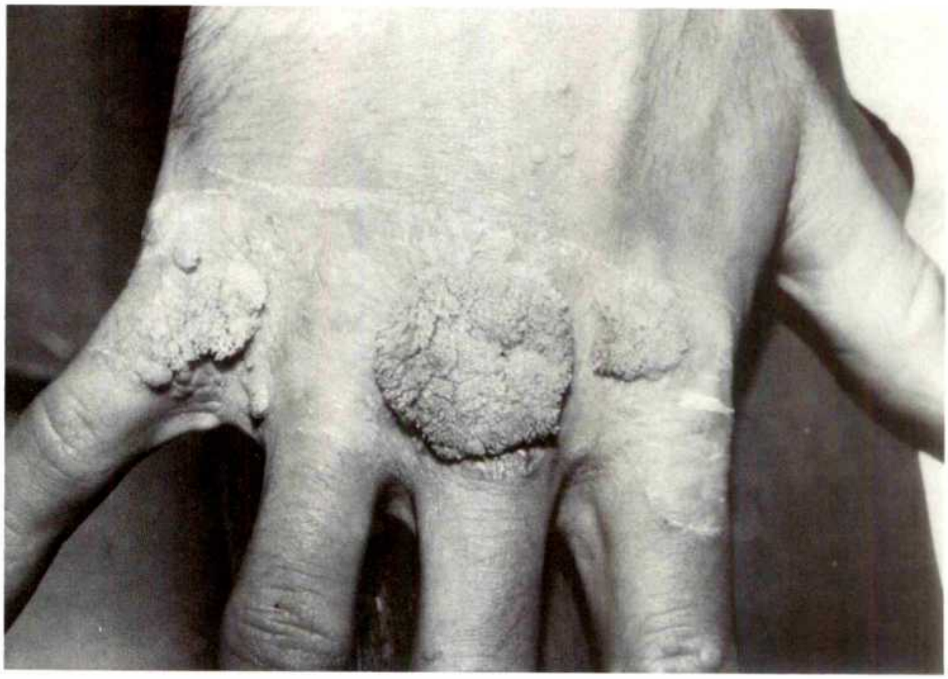

FIGURE 3. Cauliflower-like warts on knuckles prior to treatment. Still present at 12 weeks after three injections of bleomycin.

drug when injected intradermally; therefore the very small doses used in the treatment of warts are effective. It is not known how long bleomycin remains bound in the skin. Bioassay methods are complicated and although sensitive may not pick up such low concentrations (Ohnuma *et al.*, 1974). In one of our cases with large cauliflower-like warts on the knuckles (Fig. 3) there appeared to be no response to the bleomycin within the 12 weeks of the extended trial, yet 3 months later, with no further treatment, these warts had disappeared (Fig. 4) whilst all his untreated warts remained, suggesting local persistence of the drug for several weeks.

The mechanism by which bleomycin cures warts is not clear. We agree with Willoughby and others that micro-haemorrhages occur in the wart in addition to any cytotoxic or virucidal action which may occur. We are continuing histological, electron microscopic and immunological studies to try to establish the changes that occur.

The use of any agent acting upon DNA raises anxieties regarding induced chromosomal and neoplastic changes. Benedict *et al.* (1977) found that the capacity of bleomycin to transform a mouse cell line increased with concentration, with no transformation occurring at 0·1 μg/ml to a maximum of 0·5% at 2·5 μg/ml. The concentrations bear a relationship to the doses used in the treatment of neoplasms but are far above those used in the treatment of warts. The chromosomal changes in hamster cells followed an identical pattern. They noted close correlation between their studies and *in vivo* situations in experimental animals.

Since the completion of our study Shumer & O'Keefe (1983) have published the results of a somewhat similar trial. They used a larger injection dose and gave treatments more frequently. They consequently had a higher level of cure, but their findings and conclusions agree well with ours.

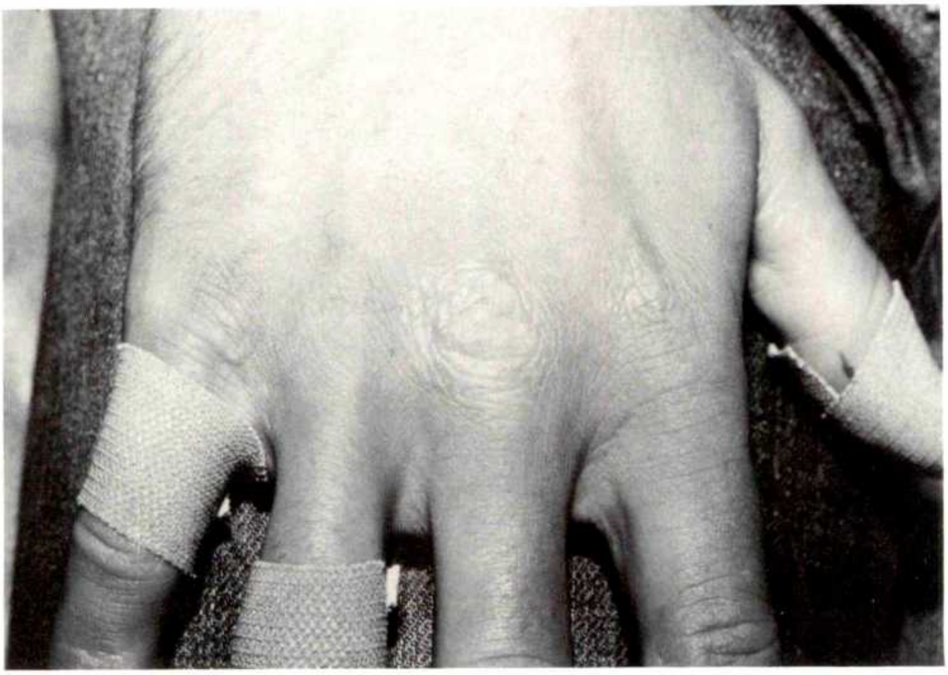

FIGURE 4. Knuckles of patient shown in Fig. 3. Clear of warts 3 months later without further treatment.

We have been particularly encouraged by the fact that eight of the twelve patients cured in the course of our trial were cured in spite of a personal or family history of endocrine or immunological disorders. The success of the alternative treatments for warts, such as DNCB sensitization and challenge, depends on the possibility of immunological response. It appears that bleomycin acts directly and is therefore particularly valuable when the immune response is diminished or absent.

We consider the intralesional injection of bleomycin a reliable treatment for resistant warts, which is safe in the short term and probably in the long term also. It remains for this form of treatment to be licensed for the treatment of warts in this country and for economically acceptable sized vials of the powder to become available.

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Oral spironolactone improves acne vulgaris and reduces sebum excretion

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SUMMARY

In a consecutive series of thirty-six male and female patients referred with severe acne, the effect of 3 months' treatment with placebo or spironolactone (50–200 mg daily) on sebum excretion and clinical and endocrine status was evaluated double-blind. Twenty-six patients completed the study. Abnormal free androgen indices were found in 27% of the original nineteen female subjects. Spironolactone reduced sebum excretion in all female subjects, but there was no correlation between sebum response and androgen status. The clinical response was dose-dependent, with maximum subjective and objective benefit when spironolactone doses of 150–200 mg were used.

Although there are several factors involved in the pathogenesis of acne, the most important phenomenon is considered to be increased sebum production. The precise biochemical control of this is uncertain, but it is known that sebaceous gland activity is increased by androgens (Pochi & Strauss, 1974). Oral cyproterone acetate has been noted to reduce sebum secretion (Hammerstein *et al.*, 1975; Ebling *et al.*, 1977), but so far no systematic studies have been performed using another anti-androgen, spironolactone, although it is has been shown to inhibit sebaceous gland activity in hamsters (Luderschmidt, Bidlingmaier & Plewig, 1982). There are isolated reports of its use in a few acne patients (Ebner, 1963; Boisselle & Tremblay, 1978).

We have therefore investigated the effects of different doses of spironolactone compared with placebo in a series of patients with acne, to determine dose-response relationships in sebum secretion and clinical effects.

METHODS

Thirty-six patients (seventeen male, nineteen female) with severe acne (aged 18–38; mean 24 years) were entered into the study, and all treatment for acne was terminated apart from the soap

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which patients were already using. No patients on oral contraceptives were included in the trial, and all female patients were advised to use other non-hormonal contraceptive precautions, if necessary.

Sebum secretion rate was measured on the forehead by the method of Strauss and Pochi, as modified by Cunliffe and Shuster (1969). Measurements were made before treatment and at 6 and 12 weeks. The acne severity was assessed by three methods. The appearance of the patients' acne was assessed and noted by the same observer (A.G.) at each visit (objective assessment). Standard monochrome photographs of the face as well as the chest and back (where applicable) were taken before treatment and at 12 weeks and evaluated 'blind' by P.W. and two observers unconnected with the study (photographic assessment). The clinical response noted by the patient was also recorded (subjective assessment) (Fig. 1). Responses were recorded as improved, unchanged or worse.

Blood samples were taken at the beginning and end of the trial, for plasma testosterone (T), assayed by the method of Collins *et al.* (1972), and sex hormone binding globulin (SHBG), assayed by the method of Iqbal and Johnson (1977). Free androgen index (F.A.I.) was calculated as the ratio (T/SHBH) × 100 (Carter *et al.*, 1983). Serum electrolytes, urea and creatinine were measured in all patients before and at the end of the study. All female patients were asked to document their menstrual cycles.

For the study, patients were randomly allocated, by sealed envelope, to the following five groups: placebo, spironolactone 50 mg, 100 mg, 150 mg or 200 mg. The trial was performed

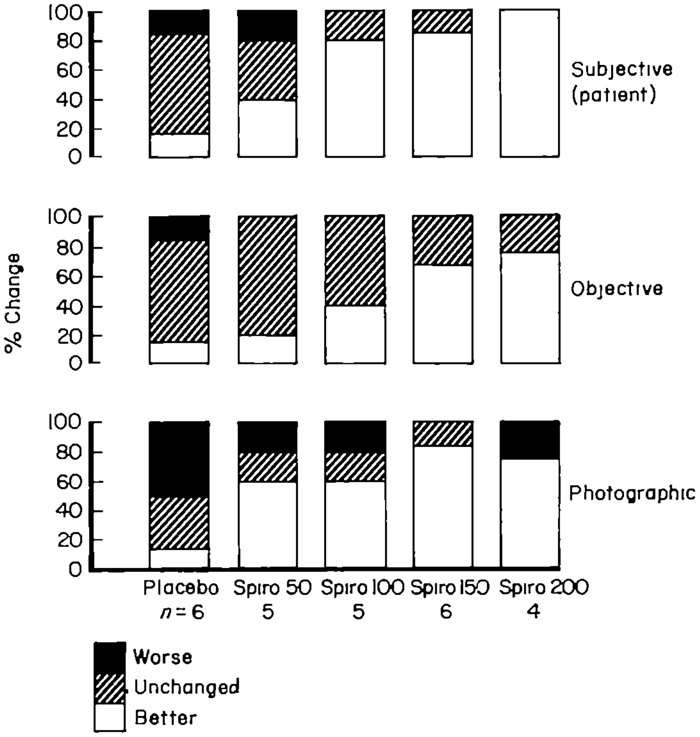

FIGURE 1. Subjective and objective responses of acne in five treatment groups: photographic records were examined by three independent assessors.

double blind to all investigators, only the pharmacist being aware of the dosage allocation. The results are presented, unless otherwise stated, as mean ± s.e.m. and the significance was determined, using paired or unpaired Student's *t*-test, as appropriate.

RESULTS

Of the original thirty-six patients, twenty-six (thirteen male, thirteen female) completed the 3-month trial. One patient discontinued 'treatment' after 1 month because she became extremely depressed about her skin, which had not improved. Another patient was withdrawn from the study by the general practitioner. The remaining eight did not return after the initial baseline studies had been performed.

Clinical changes

These are represented in Fig. 1 as histograms: both males and females were combined, because of small numbers. Good agreement was demonstrated between all three methods of assessment, doses of 150 and 200 mg appearing to confer the greatest benefit. In six of the nine females, and five of the six males receiving 100 mg or more spironolactone daily, consistent improvement was recorded by the three modes of assessment.

Adverse effects

Seven of the twelve females on active therapy noted minor increased frequency of periods, none of which was of major concern to the patient or a reason for discontinuing therapy. A transient diuretic effect was noted in two of the females. Gynaecomastia in males was not seen during the short treatment periods in this study and no significant changes were seen in serum electrolytes.

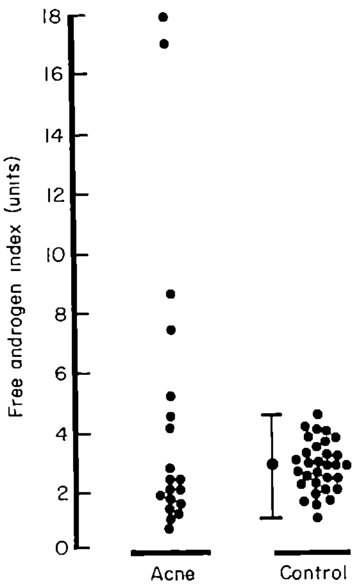

FIGURE 2. Distribution of baseline free androgen index in nineteen female acne patients compared with age-matched control population, $n=32$ (mean age 25 ± 5 years). Distribution bar is mean ± s.d.

Endocrine results

The baseline results for free androgen index in the female subjects are shown in Figure 2, compared with control data from an age-matched population. Five of the original nineteen female subjects demonstrated a high free androgen index; ten of the nineteen also showed a low SHBG. There was no correlation between either total testosterone or free androgen index and basal sebum secretion in either female or male subjects ($P > 0{\cdot}1$).

In response to treatment female subjects on active therapy showed a mean plasma testosterone rise from $1{\cdot}3 \pm 0{\cdot}1$ to $1{\cdot}9 \pm 0{\cdot}1$ nmol/l ($t = -4{\cdot}5$; $P < 0{\cdot}01$). SHBG fell from $55{\cdot}0 \pm 9{\cdot}6$ to $45{\cdot}3 \pm 5{\cdot}7$ nmol DHT bound/l ($P > 0{\cdot}1$), and F.A.I. rose from $3{\cdot}3 \pm 1{\cdot}1$ to $5{\cdot}0 \pm 1{\cdot}0$ ($P > 0{\cdot}05$). Male patients on active therapy showed no significant changes in endocrine values: serum testosterone $25{\cdot}0 \pm 6{\cdot}3$ cf. $23{\cdot}8 \pm 3{\cdot}0$ nmol/l, SHBG $33{\cdot}5 \pm 5{\cdot}1$ cf. $29{\cdot}0 \pm 3{\cdot}2$ nmol DHT bound/l, and F.A.I. $81{\cdot}1 \pm 19{\cdot}3$ cf. $83{\cdot}3 \pm 6{\cdot}3$ (P all $> 0{\cdot}1$).

Sebum excretion rate

Precision of duplicate measurement (right and left forehead), was considered satisfactory with an average difference from the mean of ±7·0%. Mean sebum excretion rate fell in both sexes (Fig. 3). In the females, sebum secretion rate fell from $4{\cdot}7 \pm 1{\cdot}7$ to $1{\cdot}0 \pm 0{\cdot}2$ μg/cm^2/min

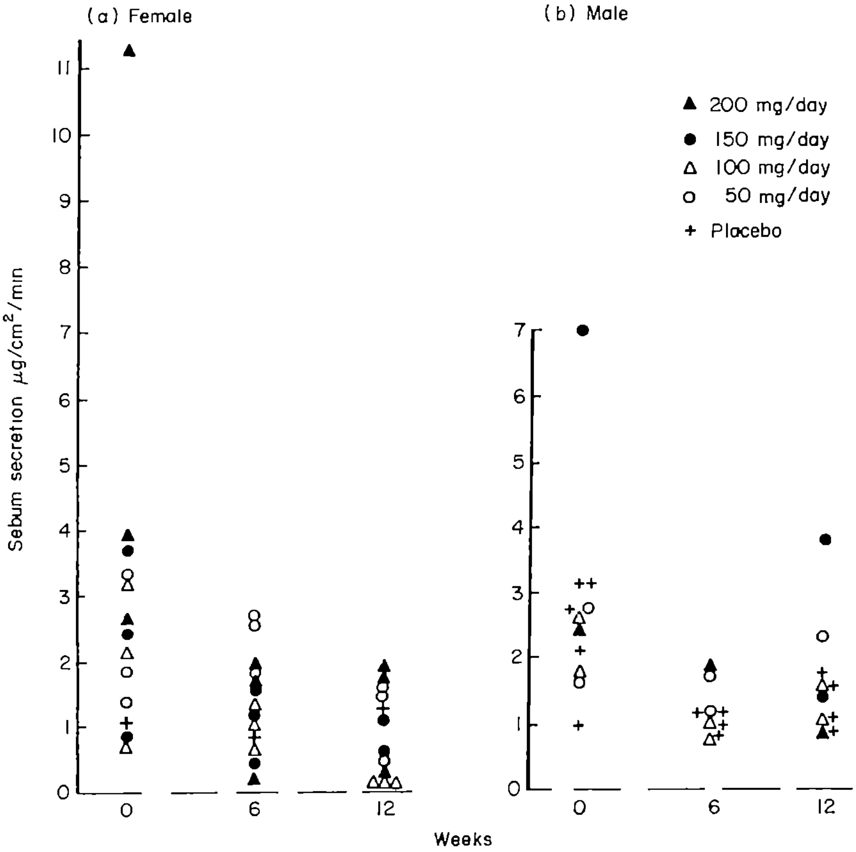

FIGURE 3. Sebum secretion rates in females (a) and males (b) completing the 3-month study: two male subjects who did not complete the full series of sebum studies have been omitted.

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($t = 2{\cdot}17$; $P < 0{\cdot}025$) for the combined dose schedules. In males the sebum secretion rate fell in the treated groups from $2{\cdot}8 \pm 0{\cdot}7$ to $1{\cdot}8 \pm 0{\cdot}3$ $\mu g/cm^2/min$ ($t = 2{\cdot}47$; $P < 0{\cdot}01$). Male placebo subjects also fell by the end of the study ($2{\cdot}5 \pm 0{\cdot}6$ to $1{\cdot}3 \pm 0{\cdot}5$; $t = 1{\cdot}5$; $P > 0{\cdot}05$). The regrettable absence of a significant number of placebo female subjects was due to selective drop-out of this group of subjects after the initial baseline studies were completed.

DISCUSSION

There is now considerable data to suggest that androgens are important in the pathogenesis of acne (Lawrence, Katz & Robinson, 1981) especially in the more severe forms (Marynick *et al.*, 1982). This idea is supported by the present study, the five subjects with the highest free androgen levels having the clinically more severe grades of acne.

Anti-androgens offer promise in the treatment of acne vulgaris. Oral cyproterone has been used in several studies (Hammerstein *et al.*, 1975), but side-effects may limit its use (Braendle *et al.*, 1974). Topically applied anti-androgens have also been used (Fayolle, 1975), but results have not been impressive.

Spironolactone is a widely used drug in the treatment of hypertension and fluid retention states. Well-known side-effects include gynaecomastia in men and menstrual disturbance in women, reflecting a series of endocrine changes related to inhibition of androgen binding to receptors, as well as increased oestrogen secretion (Tidd *et al.*, 1978). In this study the drug was well tolerated at all doses, only a transient diuresis being noted by some patients.

Although the number of individuals studied in this trial was small, there was a very marked improvement in three of the four patients who received 200 mg of spironolactone daily. These particular patients had severe cystic acne (two females and one male), and had failed to respond to many other forms of therapy, including antibiotics and a variety of topical applications. An overall tendency to improve was also noted in patients taking 150 mg of spironolactone daily, and the degree of concurrence between the subjective (patient), objective (A.G.) and photographic (three independent observers) assessments suggests a significant effect of this drug in acne. Other workers have reported subjective response of acne to spironolactone therapy (Boisselle & Tremblay, 1978) and the drug clearly needs extended evaluation, probably at the maximum dosage of 200 mg daily used in the present study.

It was noted that five patients in the present series had free androgen indices above the normal range: the behaviour of three of these who were in the active treatment group could not be distinguished from those with androgen levels within the normal range. However, the sample numbers were small, and further studies may demonstrate a selective benefit in individuals with high plasma androgen levels.

The fall of sebum excretion into the normal range in a high proportion of the subjects could be attributed to spironolactone itself. However, in view of the changes demonstrated in males given placebo, it is possible that part of the observed fall in sebum secretion rate was due to unrelated factors although all known contributory factors to sebum secretion (such as ambient temperature) were kept constant. Nevertheless, the mean 80% fall in sebum secretion seen in the women in the present study exceeds that recorded with most other forms of therapy, including combination oestrogen–glucocorticoid regimes (Saihan & Burton, 1980).

The changes in androgen metabolism were surprising. In a separate study from this hospital in patients with hirsutism and androgen excess treated with spironolactone, a mean fall in F.A.I. was observed over a 6-month period. Indeed, such a fall was noted in two of the three subjects with initially elevated levels, who were receiving active therapy. The overall mean rise in F.A.I.

seen in female subjects in the present study may reflect a stimulation of negative feedback control of testosterone secretion by the antiandrogenic effect of spironolactone.

We conclude from the results of this study that spironolactone may be useful treatment for acne. Currently, an additional double-blind study in a larger series of female patients employing a dosage of 200 mg daily is being undertaken to determine the full spectrum of response, and the relationship (if any) to baseline androgen metabolism. The usefulness in males is limited by gynaecomastia and impotence which is common with prolonged treatment (Greenblatt & Koch-Weser, 1973).

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Two cases of distinctive exudative discoid and lichenoid chronic dermatosis of Sulzberger and Garbe responding to azathioprine

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SUMMARY

We describe two cases of distinctive exudative and lichenoid chronic determatosis of Sulzberger and Garbe which showed rapid resolution with azathioprine therapy, having failed to resolve with oral prednisolone.

Since first described by Sulzberger and Garbe (1937), only eighty-three cases of discoid and lichenoid dermatosis have been reported with sufficiently clear and detailed descriptions to leave little doubt as to the diagnosis. The condition is, however, well-recognized in the United States of America, and Sulzberger (1979) reports that approximately one-third of over 300 dermatologists attending an American Academy of Dermatology symposium on Classics in Dermatopathology had seen cases of Sulzberger–Garbe disease. The reported cases show a distinct predilection for middle-aged Jewish males. It is surprising that the condition has been diagnosed rarely outside North America. When practising in Zurich in the late 1920s, Sulzberger saw several patients with what was subsequently to be recognized as Sulzberger–Garbe disease. There have been other cases reported in central Europe (Sulzberger, March & Brunauer, 1965), but the discrepancy between the recognition of the disease in the United States and elsewhere remains unexplained. This is the first report of the occurrence of this dermatosis in the United Kingdom and the first report of the effectiveness of azathioprine in this condition.

Case 1

A 77-year-old white, non-Jewish, British male presented with a widespread, intensely pruritic, skin eruption. The lesions first appeared on his abdomen and right shin. One month after the onset he was seen by a dermatologist who considered these lesions to be suggestive of lichen

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planus, but also commented that there was widespread secondary eczematization. The skin lesions were intensely pruritic and failed to improve with betamethasone valerate cream, initially in 20% dilution and subsequently undiluted. Eight months later he was re-referred. The pruritus continued but the lichenoid lesions had resolved and he now had extensive areas of eczematization and excoriation. Trimeprazine orally together with continuance of his topical steroid afforded no symptomatic relief. A biopsy revealed an intense perivascular infiltrate of monocytes, plasma cells, histiocytes, eosinophils and polymorphonuclear leukocytes. No giant cells were present. The blood vessels showed marked endothelial swelling. His topical steroid therapy was changed to clobetasol proprionate ointment and he was also prescribed arachis oil baths. His pruritus continued unabated.

He was referred to our department in December 1980. His skin problems had by then been present for approximately 4 years and the pruritus was so severe that he described himself as 'almost suicidal' at times. When first seen he had a rather streaky, lumpy, lichenoid eruption on his limbs. A further course of clobetasol proprionate ointment, together with hydroxyzine hydrochloride orally, was prescribed. When seen 2 weeks later the skin lesions consisted of areas of nodular prurigo, patches of erythema and scattered lichenified plaques. There was no obvious clinical diagnosis at this stage; lichen planus, mycosis fungoides, and a pre-bullous erythema were considered but did not fit the clinical picture. He was admitted to hospital for further assessment. Whilst in hospital his erythematous lesions disappeared, using only betamethasone valerate cream in 25% dilution. The lesions rapidly recurred when he left hospital. Biopsies were taken from both the erythematous and lichenified lesions; both showed the same features as in the earlier biopsy. Immunofluorescent studies revealed a very faint intermittent deposit of complement C3 at the basement membrane zone. He was extensively investigated for internal pathology associated with his skin disease but no abnormalities were found apart from an eosinophilia of 6% of a total white blood cell count of $7 \cdot 4 \times 10^9/l$. The possibility that he had an unusual form of vasculitis led to a trial of dapsone 50 mg twice daily but his skin condition worsened whilst on this drug and it was therefore discontinued after 2 weeks.

He was discharged from hospital using 25% betamethasone valerate topically, but his skin condition worsened and topical steroids of increasing potency were substituted, and by 1 month after discharge he was again using clobetasol proprionate ointment. At this stage he had extensive areas of eczematous skin and was commenced on prednisolone 20 mg daily. As he had a history of duodenal ulceration cimetidine was also prescribed. There was no improvement in his dermatosis and he reported that he had developed severe sweating at night, not associated with fever. There was no evidence of tuberculosis and the history therefore raised the possibility of an underlying lymphoma. Two further skin biopsies revealed the same changes as previously seen. He had no hepatosplenomegaly or palpable lymphadenopathy. His chest X-ray was normal. Computerized axial tomography of his abdomen was performed but revealed no abnormalities. The diagnosis of lymphoma was therefore abandoned. The skin eruption was now very widespread sheets of erythema, the lichenified lesions having resolved. His prednisolone was continued in a dose of 20 mg daily, but even when combined with topical clobetasol proprionate ointment under occlusion and hydroxyzine hydrochloride by mouth there was no sustained improvement.

There was an apparent response when he was first admitted to hospital for these investigations but new lichenoid and erythematous lesions appeared within 3 days. During his hospitalization the character of his skin lesions was seen to change within 2 or 3 days, sometimes looking lichenoid, sometimes eczematous and sometimes showing sheeted erythema. Ten weeks after the prednisolone was commenced a diagnosis of Sulzberger–Garbe disease was made. A review

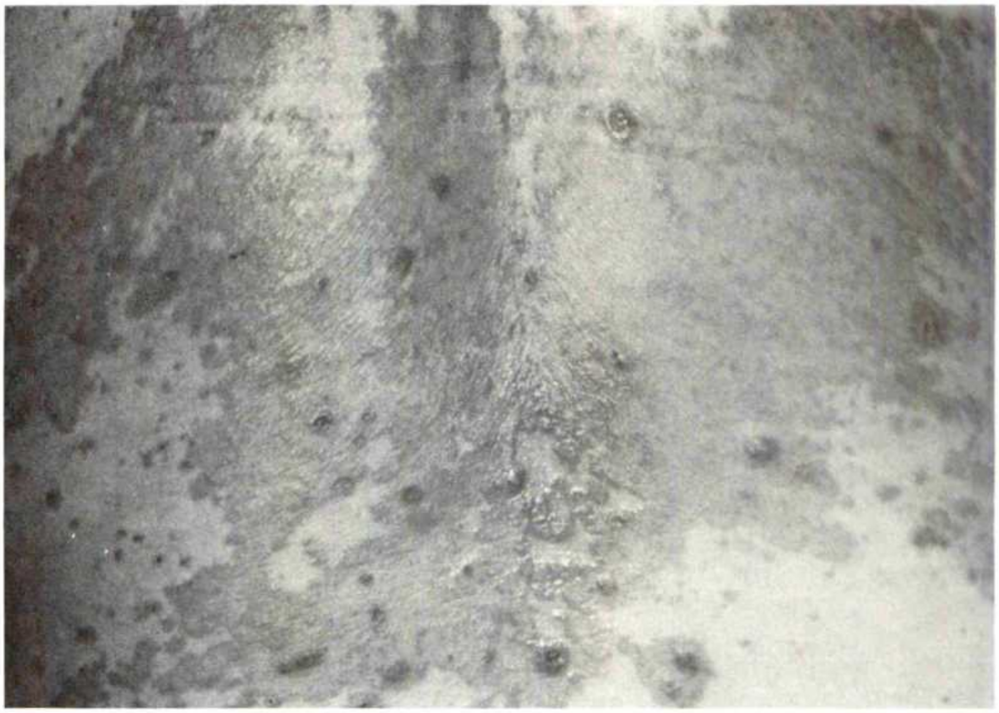

FIGURE 1. Case 1. Sheeted erythema and lichenoid lesions on back.

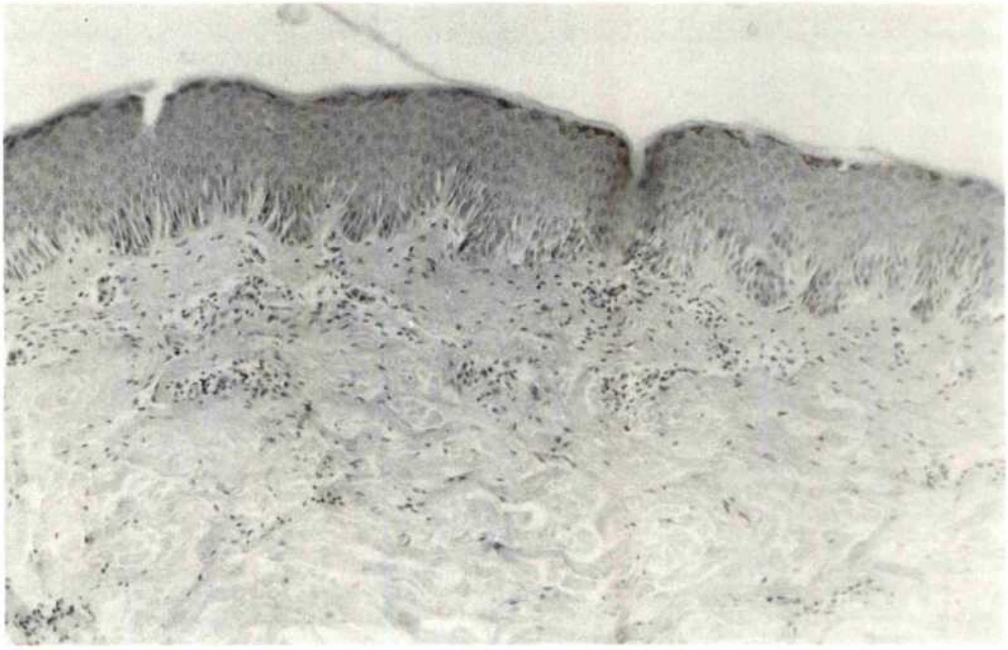

FIGURE 2. Histopathology of erythematous lesions shown in Fig. 1. Perivascular infiltrate and swollen endothelium of small blood vessels. (Haematoxylin & eosin, × 100).

of his clinical and laboratory findings showed them to be consistent with this diagnosis. As he had failed to show the anticipated improvement with corticosteroids, azathioprine 50 mg twice daily was added. Within 2 weeks he was almost clear of skin lesions and was free of pruritus. The dose of azathioprine was increased to 125 mg daily and after a further 2 weeks he had no visible skin lesions and remained free of pruritus. The dose of prednisolone was reduced by 5 mg at monthly intervals and the azathioprine continued. Over the next year the dose of azathioprine was gradually reduced and his dermatosis remained in remission 11 months after ceasing therapy.

Case 2

A 66-year-old white, non-Jewish British male presented with a 2-month history of a widespread intensely pruritic skin eruption which consisted of grouped papules on his left knee, midriff and shoulders. He attributed the onset to insect bites as it followed an episode of strenuous gardening. The lesions resembled insect bites. No puncta were present in the papules, no contacts had similar symptoms and no parasite was identifiable. A biopsy of a papule on the right flank was compatible with papular urticaria, showing parakeratosis, intracellular oedema, spongiotic vesicles, dermal oedema and a focal perivascular dermal infiltrate of mixed cellularity but including numerous eosinophils. No giant cells were present. Dermal vessels were dilated and prominent. A blood sample revealed an eosinophilia of 8% of a total white cell count of $8 \cdot 19 \times 10^9/l$.

A diagnosis of papular urticaria was made and he was treated with oral chlorpheniramine and topical crotamiton cream with no improvement. Brompheniramine maleate and crotamiton-hydrocortisone cream were substituted but produced no improvement. Over the next 6 weeks the intensely irritating eruption continued but developed a migratory pattern, affecting different parts of the body at each assessment. The papules became larger and were associated with dermographism. A 2-week course of terfenadine and a 2-week course of promethazine hydrochloride together with topical clobetasol proprionate cream produced no improvement.

In view of the continuing severe pruritus and lack of response to therapy, a 3-week course of oral prednisolone was given, commencing at 30 mg daily and reducing by 5 mg every third day. there was no symptomatic improvement and by this stage, 4 months after onset, the lesions were no longer papular, but consisted of eczematous plaques and widespread, erythematous, sheet-like lesions. Clinically both a pre-reticulosis and an unusual eczematous reaction were considered. A further biopsy was performed. This revealed the same features as in the original biopsy but with increased spongiosis and tendency of the inflammatory cells to spread into the epidermis. The number of eosinophils in the infiltrate was reduced. A further course of prednisolone gradually reducing from 30 mg to zero over a 6-week period was totally ineffective and whilst on this drug he developed infiltrated, lichenified plaques on his trunk and limbs. Clinically the lesions were now very strongly suggestive of lichen planus. He had no oral or penile lesions at any stage. A third biopsy was taken from a lichen planus-like area and histological examination revealed the same changes as in the second biopsy. A diagnosis of Sulzberger–Garbe disease was made at this stage and 1 week later, 4 months after stopping the prednisolone, he commenced treatment with azathioprine 50 mg three times daily. When seen 2 weeks later he reported a dramatic diminution in pruritus for the first time in approximately 8 months. The skin lesions were no longer visible. After 6 weeks he remained free of pruritus and skin lesions. The dose of azathioprine was gradually reduced and after 6 months it was discontinued. There has been no recurrence of his dermatosis in the following 7 months. Review of all biopsies after the diagnosis of discoid and lichenoid dermatosis was suggested showed that the changes were consistent with those previously described in that condition (Sachs & Kirsh, 1947; Sulzberger, 1979).

DISCUSSION

In Case 1 the following were considered prior to a diagnosis of Sulzberger–Garbe disease: lichen planus, nodular prurigo, eczema, mycosis fungoides, pre-bullous erythema, vasculitis and a lymphoma. In case 2 nodular prurigo, dermographism, a pre-reticulosis, eczema and lichen planus were considered. The rapidly changing character of the lesions and the biopsy findings were such that each of these diagnoses could be ruled out, frequently on both clinical and histological grounds. At no stage were any of these possible diagnoses supported by both clinical and histological evidence. It is one of the characteristic features of Sulzberger–Garbe disease that many other diagnoses have been considered prior to diagnosis (Sulzberger, 1979); our experience certainly supports this.

There is no single diagnostic criterion for Sulzberger–Garbe disease, either clinically or histologically. Sulzberger (1979) lists the following characteristics:

(1) A distinct predilection for middle-aged males (mainly Jewish).
(2) Consideration of many diagnoses which could all be ruled out eventually.
(3) Rapid changes from slightly elevated discs to exudation to lichenification and *da capo*.
(4) A high prevalence of penile lesions.
(5) Eventual clearing of discoid, exudative and lichenoid lesions.
(6) Blood eosinophilia.
(7) Histologically, cloudy swelling of cutaneous blood vessels and perivascular infiltrates of lymphocytes, polymorphonuclear leukocytes, including some eosinophils, and plasma cells. Occasional epithelioid cells but no giant cells are seen.

Neither of our patients was Jewish and neither developed penile lesions. However, both of them closely fit the clinical and histological patterns of Sulzberger–Garbe disease and cannot be diagnosed as any other recognized entity. We thus feel that our diagnosis is justified in these cases. In common with previously reported cases the dermatosis was intensely pruritic, had discoid, lichenoid and erythematous lesions which coexisted or rapidly changed from one form to another; was associated with a blood eosinophilia; ran a chronic course; improved with change of environment and had the histological features listed above.

A feature of our patients was the lack of response to oral corticosteroids. Sulzberger (1979) has stated that discoid and lichenoid dermatosis often responds to small doses of oral corticosteroids, but that this suppressive treatment must be continued until self-healing occurs. Such self-healing is a feature of this condition but is a gradual process during which the pre-existing skin lesions are superseded by small, deep-seated, urticaria-like lesions. The complete resolution of the dermatosis so soon after the introduction of azathioprine in both of our cases was very striking. This suggests that the drug may have been responsible for the clearance, even though azathioprine normally takes several weeks to act in other conditions. We suggest that azathioprine be considered in the management of future patients with this dermatosis.

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Case Reports

Bullae in skin grafts

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SUMMARY

We describe eight patients with grafted leg ulcers, in whom subepidermal bullous lesions appeared after 4–8 weeks either at the donor or the grafted site. The blisters were seen in the centre as well as just outside the graft. They remained for some days and then disappeared. Histology revealed a normal epidermis and a dermis with a moderate lymphocytic infiltration around the capillaries. No clinical signs of pemphigoid were found and friction does not seem to have been a contributing factor. We have at present no explanation for the appearance of the blisters.

Two patients who developed blisters at the donor site of split-thickness skin grafts have recently been reported (Barker & Cotterill, 1980; Berman, 1982). Although bullae are not usually mentioned in reports on skin grafts (Millard, Roberts & Gatecliffe, 1977) such blisters may not be unusual (Agris, 1982). The bullae appear 3 weeks after the grafts are taken, and disappear spontaneously after several weeks. We report here the formation of blisters at both the donor sites and the grafted sites.

PATIENTS

The history of the patients is listed in Table 1. After grafting the patients stayed in bed for 10–14 days with bandages on both the donor and the grafted site. Thereafter the bandages were removed and the patients were allowed to walk around without any bandages or elastic stockings. None of the patients were exposed to sunlight or had taken photosensitizing drugs.

Bullae were seen at the site where the grafts had been taken, and at the grafted site the blisters were seen in the centre as well as just outside the graft (Figs 1 and 2). Sometimes several bullae appeared at the same time. They remained for some days and then disappeared without recurring. There were no signs of bacterial infection.

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TABLE 1. Patients with grafted leg ulcers showing bullae. The donor site was always the upper leg and the grafts were on the lower leg

No.	Age (years)	Sex	Type of graft	Weeks between graft and appearance of bullae	Site of bullae
1	90	F	Pinch graft	2	Graft
2	81	M	Pinch graft	2	In and around graft
3	75	M	Pinch graft	8	Donor
4	79	F	Dermatome	7	Donor
5	85	F	Pinch graft	4	In and outside graft
6	72	F	Pinch graft	4	Outside graft
7	82	F	Pinch graft	104	Spontaneous in old graft
8	70	M	Pinch graft	8	In graft

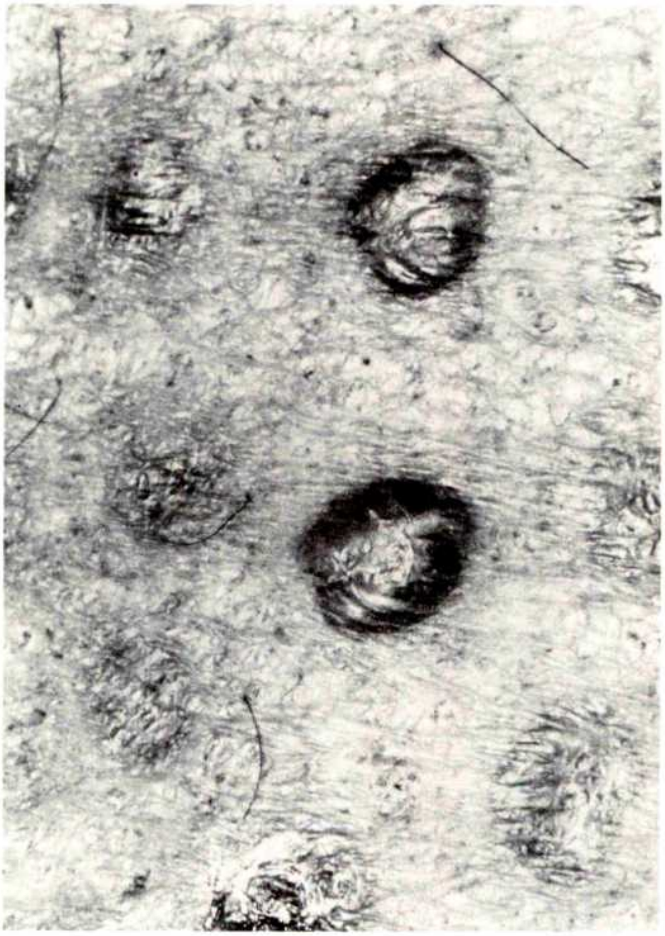

FIGURE 1. Bullae on the donor site.

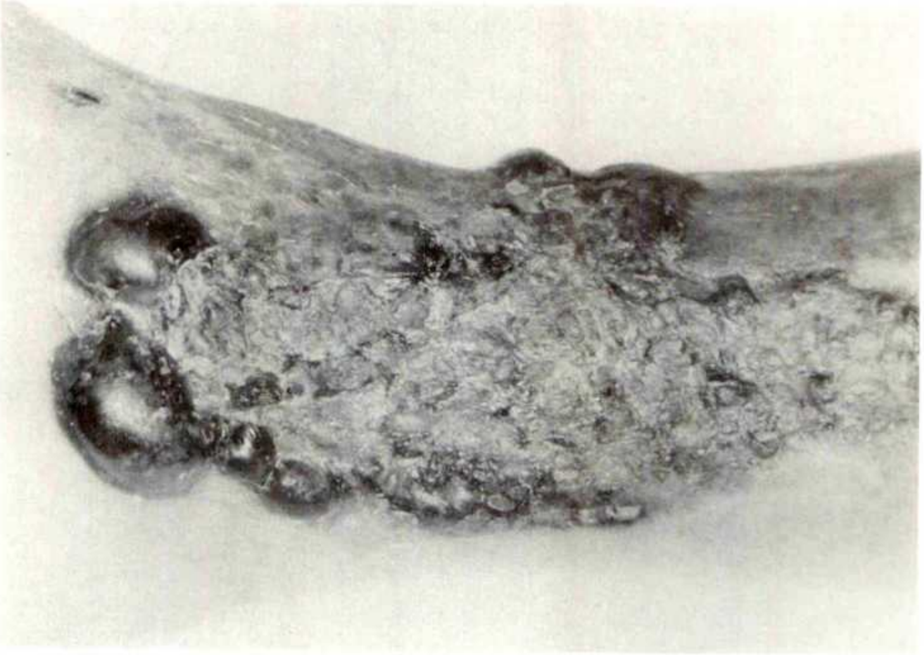

FIGURE 2. Bullae at the periphery of the grafted site.

HISTOLOGY

A subepidermal blister containing some fibrin structures was seen in all cases (Fig. 3). The epidermis appeared normal without signs of acantholysis. In the dermis the capillaries were dilated with a slight surrounding infiltrate of lymphocytic cells. There were a few or no leukocytes in this infiltrate. There was oedema of the deeper part of the dermis.

DISCUSSION

Friction from clothes and inflammation resulting from stasis of lymph are possible factors in pathogenesis of the bullae formation both in the grafts and in the newly formed epidermis. Deposition of fibrin, as occurs in frictional lesions (Wong & MacDonald 1982), could also be a contributing factor to the bullae formation. In our patients we had no evidence that friction was involved. A change in posture from the horizontal to the vertical position could be an explanation in patients number 1 and 2, but not in the others, where the bullae appeared several weeks after grafting.

Recurrent bullous eruptions on the lower legs have been described in elderly patients with vascular insufficiency (Frain-Bell, 1959; Bjornberg & Mobacken, 1976). Frain-Bell (1959) assumed that they were due to bullous eczema whereas Bjonberg and Mobacken (1976) in most of their patients found no signs of eczema or trauma. They found no signs of bullous pemphigoid, and circulating antibodies against basement membrane could not be demonstrated. In our patients, and those reported with bullae in the donor site (Barker & Cotteril, 1980; Berman, 1982), there were no immunological signs of pemphigoid. Bullous pemphigoid can be limited to the lower extremities (Person, 1983). In one study Ullman, Halberg and

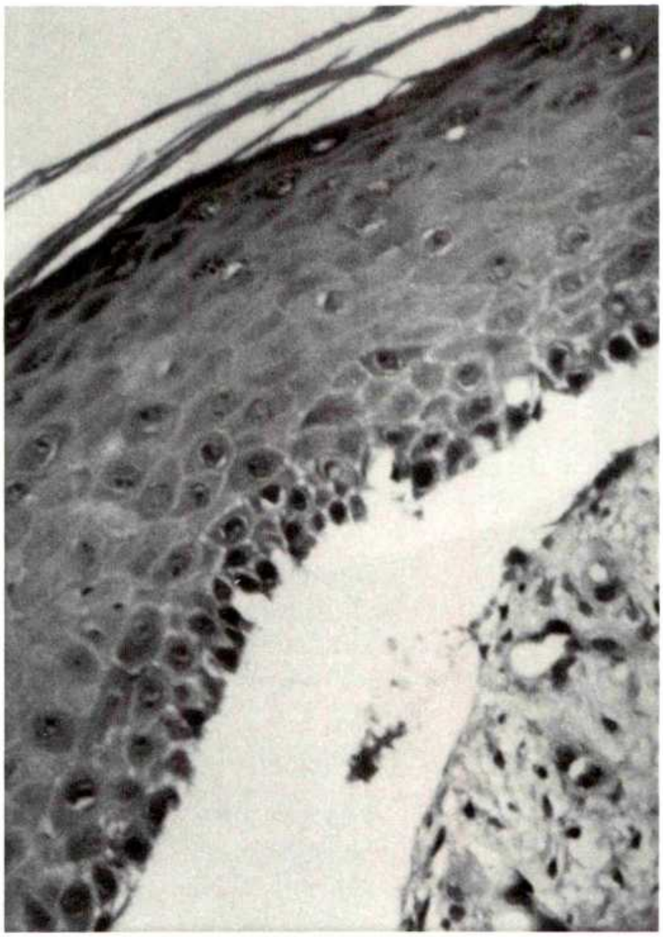

FIGURE 3. Histology of subepidermal bulla showing a normal epidermis, with dilated capillaries in the dermis.

Nielsen (1975) found four out of fifty-eight patients with leg ulcers had an anti-basement membrane antibody in the serum. In our study we have not looked for pemphigoid antibodies in the sera, but none of our patients had clinically any signs of active localized bullous pemphigoid. Pemphigoid antigen is believed to be present when epidermal cells migrate over a wound and probably influences basal cell attachment, whereas collagen IV and laminin lag behind (Stanley, 1982). A disturbance in these factors could be a possible clue to the blister formation.

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Bullous pyoderma gangrenosum and multiple myeloma

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SUMMARY

The previously unrecorded association of superficial bullous pyoderma and IgG-producing multiple myeloma is described. A rapid response of the cutaneous manifestations was achieved by treatment of the malignant paraproteinaemia.

Pyoderma gangrenosum may be associated with either benign monoclonal gammopathy or multiple myeloma (Callen & Taylor, 1978; Holt, Davies & Saunders, 1980). In all the reported cases, IgA has been the predominant immunoglobulin involved, and the pyoderma is not usually of the superficial bullous type. We describe a case which appears to be the first report of superficial bullous pyoderma gangrenosum associated with multiple myeloma producing an associated abnormal immunoglobulin of the IgG class.

CASE REPORT

In 1978 the patient, a 56-year-old woman, complained of tiredness and lethargy. The only abnormal investigations at this time were an eosinophilia of 8%, an erythrocyte sedimentation rate (ESR) of 36 mm/h (Westergren) and a monoclonal gammopathy. She was discharged from hospital care in January 1983 remaining well until June 1983 when she complained of weight loss and pain in the lower back and left side of the chest. Four weeks after the onset of symptoms she developed reddish-brown papules which rapidly became pustular on the face, ears, legs and arms. These lesions, initially painless, developed a violaceous appearance in the perilesional skin and increased in size to form bullae, before breaking down to leave painful ulcerated areas with undermined margins (Fig. 1). Pain was severe, necessitating narcotic analgesia.

Investigations

X-rays showed collapse of the third lumbar vertebrae and lytic areas in the left fourth rib. She was anaemic (Hb 9·4 g/dl) with an ESR of 145 mm/h. The paraprotein present at a

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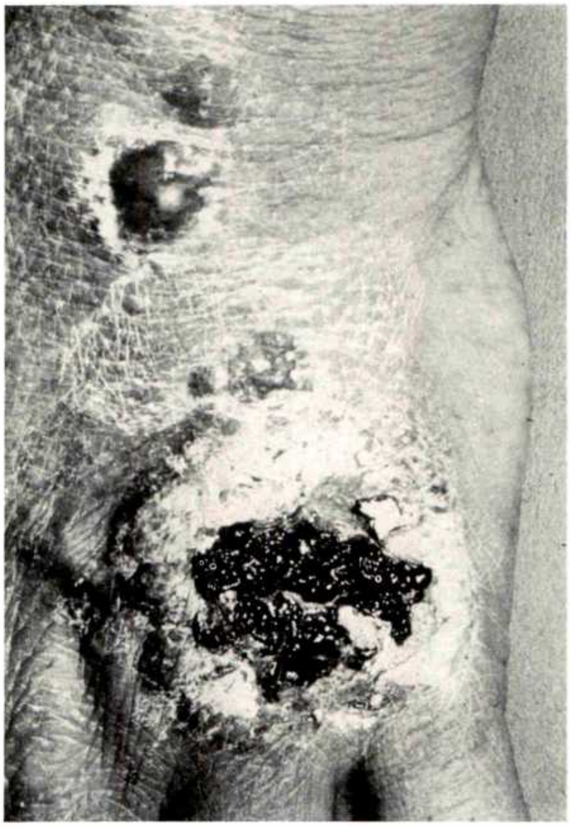

FIGURE 1. A pre-ulcerative bulla and an area of ulceration on the hand.

concentration of 55 g/l, was identified as IgG; Bence–Jones proteinuria was absent. A bone marrow biopsy showed infiltration of the marrow by malignant plasma cells, confirming the diagnosis of multiple myeloma. Renal function was minimally impaired but the blood biochemical tests were normal.

Skin biopsy of a small ulcerated lesion showed a central area of loss and necrosis of the epidermis and underlying papillary dermis. The adjacent epidermis was spongiotic with marked papillary oedema and was undermined by a dense zone of inflammatory infiltrate comprising predominantly neutrophil polymorphs with considerable leucocytoclasis. A number of eosinophils were also present in addition to scattered large hyperchromatic mononuclear cells, some of which were mitotic (Fig. 2). In the more superficial areas of the infiltrate, necrosis of dermal vessels with obvious erythrocyte extravasation was observed. More deeply, endothelial swelling with permeation of vessel walls by inflammatory cells was apparent. Intact vessels were dilated and densely crowded with neutrophils. The appearance confirmed the diagnosis of pyoderma gangrenosum.

Following treatment with melphalan and prednisolone the ulcers began to heal and the pain disappeared within 1 week. The lesions are now completely healed with residual 'tissue-paper' scarring and the patient feels well on maintenance treatment.

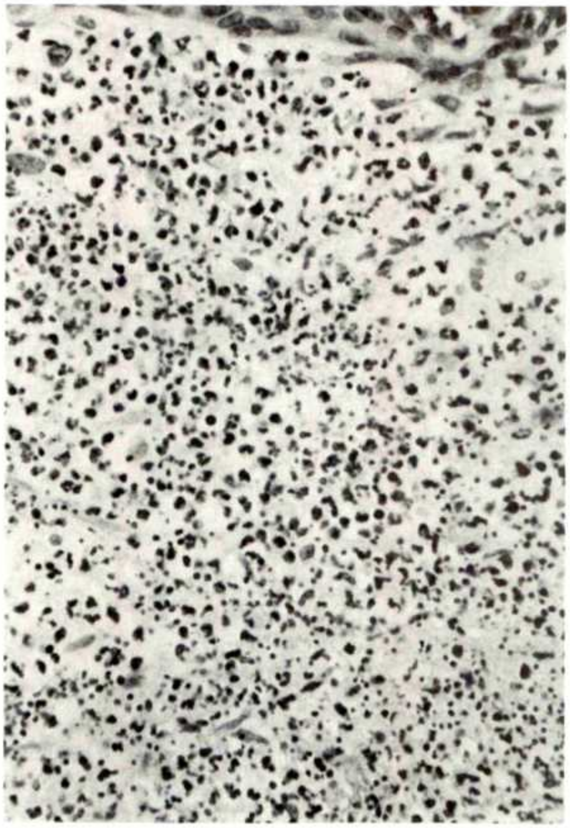

FIGURE 2. The dense upper dermal infiltrate with polymorph predominance.

DISCUSSION

The incidence of monoclonal gammopathy and pyoderma gangrenosum varies in different series. Power *et al.* (1983) reviewed sixty-seven cases and found an incidence of 11·9%. Among the reports of multiple myeloma associated with pyoderma gangrenosum (Thivolet, Pellerat & Hermier, 1964; Jablonska, Stachow & Dabrowska, 1967; Sanchez, 1980; Holt *et al.*, 1980; Powell *et al.*, 1983) the immunoglobulin involved was always of the IgA class, despite the fact that IgA accounts for less than 10% of monoclonal gammopathies (Kyle, 1978). The significance of this however is unknown. No predictive criteria with regard to malignant potential relate to paraproteinaemia (Kyle, 1978). In Kyle's (1978) series the average duration from discovery of the paraprotein to the development of myeloma was over 5 years, as in our patient. Thus the number of cases developing myeloma may easily be underestimated unless prolonged follow-up is maintained.

The superficial bullous form of pyoderma gangrenosum has been associated with acute leukaemia (Sheps, Shapero & Ramsay, 1978; Crow & Bowers, 1974) and myelofibrosis (Callen, Dubin & Gehrke, 1977). Two recent studies of pyoderma gangrenosum and Sweet's syndrome in association with myeloproliferative disorders (Caughman, Stern & Haynes, 1983; Cooper, Innes & Greer, 1983), considered that atypical bullous pyoderma gangrenosum and Sweet's syndrome are histogenetically similar neutrophil-mediated lesions, which may be difficult to distinguish in patients with myeloproliferative disorders. Atypical pyoderma gangrenosum in association with lymphoproliferative disorders, as in our case, was not recorded in either review.

Excruciating pain has been recognized as a prominent clinical feature of these cases. Rapid relief of pain and the healing of the ulcerated areas in response to treatment of the myeloma in our patient was remarkable. Resolution of ulcerated pyoderma gangrenosum has previously been observed in response to treatment of both benign and malignant gammopathies (Powell *et al.*, 1983; Moller, Waldenstrom & Zetterrall, 1978; Russell Jones *et al.*, 1983), and suggests that vigorous treatment of any associated paraproteinaemia may be appropriate in intractable pyoderma gangrenosum.

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Congenital ectopic nail of the fifth finger

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SUMMARY

A case of congenital ectopic nail (onychoheterotopia) of the fifth finger is described.

Ectopic nail is a relatively rare disorder except for the vestigial nails of the supernumerary digits (Samman, 1978). It has been reported mainly from Japan and is considered to be a hamartoma or teratoma (Ohya, 1931; Kikuchi, Horikawa & Amano, 1974).

CASE REPORT

A 9-year-old Japanese boy presented with a small, oval, keratotic papule on the palmar aspect of the tip of the left fifth finger. It was situated opposite the normal nail of the fifth finger (Fig. 1). Physical examination revealed no additional malformation, and radiography revealed normal bony structure of the affected finger. The papule had existed at birth, and grew with the same speed as the other nails, so that he had to cut it occasionally. His family history was not contributory, and no preceding injury to the portion of the finger had been noticed. The papule was removed surgically, and was shown to be connected by a yellowish tendon-like structure to the digital phalanx. Histology revealed a small but complete nail matrix with a normal nail plate (Fig. 2).

DISCUSSION

Congenital ectopic nail is a relatively rare disorder except for the vestigial nails of the supernumerary digits or onychoheterotopia associated with various skin diseases (Samman, 1978). Our case is similar to the case of onychoheterotopia reported by Ohya (1931), which was later designated as Ohya's type ectopic nail by Kikuchi *et al.* (1974, 1978). Kikuchi also suggested that this type of nail anomaly can be differentiated from congenital onychodysplasia of the index finger (COIF) described by Iso *et al.* (1969) because the localization of the ectopic nails in COIF is restricted to the dorsal aspect of the finger, and COIF has a variable clinical appearance including anonychia, micronychia, or polyonychia with or without bone anomalies of the affected finger. Similar conditions such as polyonychia associated with syndactylia and

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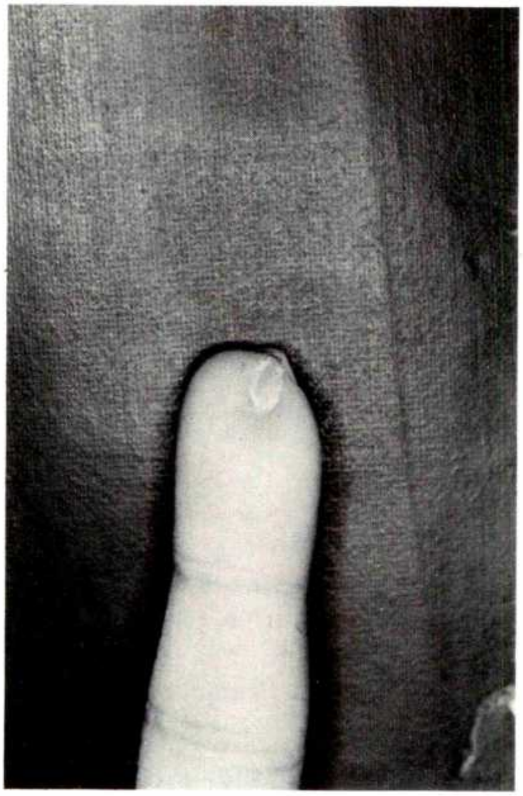

FIGURE 1. Clinical appearance of the ectopic nail.

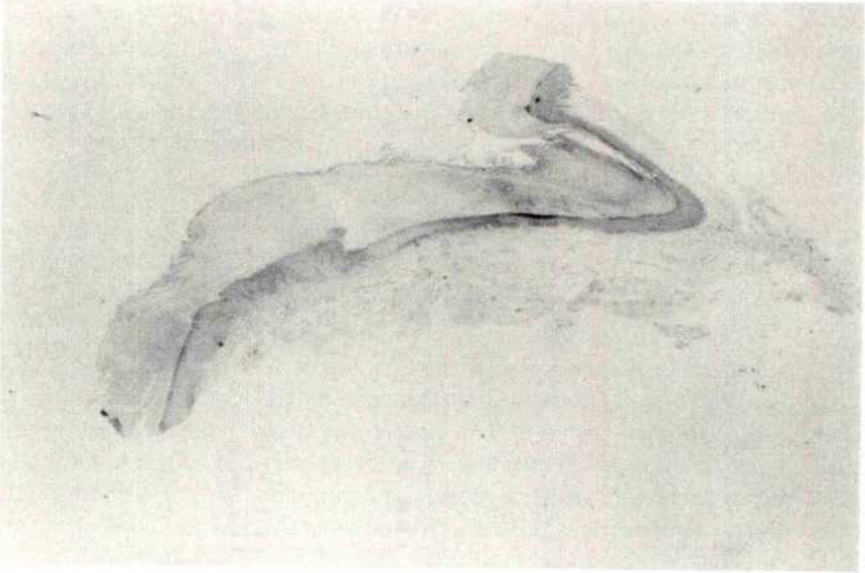

FIGURE 2. Histology of the ectopic nail. Complete nail matrix can be seen.

polydactylia, or with other skin disease such as epidermolysis bullosa, congenital ectodermal dysplasia or dyskeratosis congenita can be easily differentiated by their clinical and radiographic appearance. To the best of our knowledge, only three cases of Ohya's type ectopic nail have been reported in the literature (Ohya, 1931; Kikuchi, 1978; Aoki & Suzuki, 1984). All the patients, including our case, have been reported from Japan.

The ectopic nails were situated on the palmar aspect of the fifth finger separated from the normal nail, except for Ohya's case in which the ectopic nail was situated on the palmar aspect of the right thumb. With regard to pathogenesis, previous authors have suggested that it might develop from ectopic germ cells (i.e. a hamartoma or teratoma), though they could not exclude the possibility of a vestigial nail of an occult polydactyly. At present we prefer the theory that this type of ectopic nail is harmartomatous.

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Comment

THE AETIOLOGY OF DANDRUFF AND THE MODE OF ACTION OF THERAPEUTIC AGENTS

When I recently reviewed the literature on the aetiology of dandruff and the mode of action of therapeutic agents, I was amazed to find how overwhelming was the evidence of aetiology, and how it had been ignored because it was so well buried under a mountain of error since Malassez (1874) wrote his 'Note sur le champignon du pityriasis simple'.

I reviewed the publications exposed by a Medlars Toxline and Toxback search, including some in the increasingly overlapping area of seborrhoeic dermatitis and psoriasis, as well as a number of internal reports made available by Procter & Gamble. Because the standard of the literature is poor, with defects in design, methods, statistics and interpretation, it was necessary to build an understanding more by considering groups of papers than by individual studies, although some which have been largely ignored are well executed and have clear results. Perhaps the most alarming feature of the dandruff literature is the way in which opinion masquerades as established fact. This is due in part to articles which the novice can easily confuse with authoritative reviews, but which are used as a vehicle for poorly supported opinion and often for publication of incomplete data which would be inadmissable in a full paper: the pseudo-review becomes the source of the inadequately established idea, by subsequent quotation in conventional but uncritical reviews by others. In my view the totally discreditable hyperproliferative hypothesis and the alleged cytostatic action of anti-dandruff agents has taken hold in this way. If the reader gets a single message from this review it is 'Beware reviews'. The work divides itself into a number of topics.

1. Clinical and histopathological features

(a) Clinical. Surprisingly this is dealt with poorly, the underlying consensus being that dandruff is a scaly condition of the scalp without evidence of other skin disease either on the scalp or elsewhere. The various studies suggest a graded defect, few people having no scaling at all (Van Abbe, 1964; Leyden & Kligman, 1979). The ease of dissociating the condition from psoriasis and seborrhoeic dermatitis is stated (e.g. Ackerman & Kligman, 1969) but proof is lacking. Simple diagnostic studies allowing a panel of clinicians to examine only the scalp are required to validate diagnostic criteria. Without such evidence I see no reason to change my own conclusion that mild seborrhoeic dermatitis and psoriasis have features indistinguishable from dandruff.

(b) Histopathology. The findings are conflicting and confusing. The study of Ackerman and Kligman (1969) which gave a clear separation of dandruff from psoriasis and seborrhoeic dermatitis seems to have been retracted by Kligman and co-workers (Kligman, McGinley & Leyden, 1979), who now find features of both, apparently even on re-examination of histological material which formed the basis of their earlier report (see also Alexander, 1967; Plewig & Kligman, 1970). The thickness of the stratum corneum has likewise led to much confusion, and it is not absolutely clear that Kligman's group were not confused about their findings themselves. Thus Ackerman & Kligman (1969) report that fewer cellophane tape-strips are required to remove all scale in normal subjects and that this could mean either a thinner stratum corneum or that more comes away at each strip. Yet subsequently Kligman *et al.* (1979) say that the original cellophane tape-stripping experiment 'indicates that the stratum corneum has fewer

layers in the case of dandruff'. Unfortunately this confusion has been picked up and amplified in reviews (e.g. Van Abbe *et al.*, 1981), although it is self-evident as well as clear from the original study (Ackerman & Kligman, 1969) that the scaly stratum corneum must be thicker than normal but that when the thick scales come off what is left is thinner than normal. In summary, the published evidence is totally inadequate on whether or not dandruff has a specific histopathological appearance and whether it can be dissociated from mild seborrhoeic dermatitis and psoriasis on clinical or histopathological grounds.

2. Clinical assessment and objective measurement

(a) Clinical assessment. Methods range from an overall assessment to grading areas of the scalp (Van Abbe, 1964), but without essential preliminaries such as observer error and reproducibility, especially by those using ten clinical grades (e.g. Kligman *et al.*, 1979), an unnecessary if not impossibly large number. There is inadequate data on the optimum time for making a clinical assessment after the last shampoo although it is critical. It is essential to establish the rate of reaccumulation of scales in relationship to severity, and if a single observation of clinical grade is to be made for all cases regardless of severity, it must be done whilst reformation of scale is linear. Likewise the effects of greasiness, lighting and other obvious variables need control studies. Unresolved technical problems may therefore explain the considerable differences e.g. between Van Abbe *et al.* (1981) and Kligman *et al.* (1974) on seasonal and day-to-day variations and even between workers from the same laboratory: thus some of the findings in Plewig and Kligman's 1969 paper are more in keeping with Van Abbe *et al.* (1981) than with Kligman *et al.* (1974). Finally, with few exceptions, the statistical analysis varies from the dull to the dubious and few therapeutic studies are double blind.

(b) Objective measurement. There are two main methods: (i) scale weight after separating by vacuuming, brushing or combing (e.g. Vanderwyk & Roia, 1964); (ii) corneocyte count after localized surface scrubbing (e.g. Leyden, McGinley & Kligman, 1976). Both are equally poorly validated, and reproducibility and relationship to clinical grading have not been adequately established.

The larger surface area used in (i) is likely to give more accurate results than (ii) because the corneocytes come away in clumps and because of regional variation. Method (ii) is also likely to give an underestimate because adequacy of separation of individual corneocytes for counting is critically influenced by clump size. In general the literature suggests that clinical appearance correlates better with corneocyte weight than count, and in a recent discussion of corneocyte counts Kligman *et al.* (1979) say 'it is impossible to decide whether or not a proband has dandruff; the individual variations [in corneocyte counts] are too great'. That this admission invalidates most of the work of Kligman's group seems poorly understood because earlier in the same paper the authors claim 'we have developed such a technique [for the objective measurement of dandruff]: corneocyte counting'. In future studies it would be desirable to relate scale accumulation to a time as well as an area base.

A surprising and ubiquitous analytical weakness concerns the use of group means instead of analysing sequential changes in individuals (e.g. Imokawa, Shimzu & Okamoto, 1981). There is also a conversion to log-corneocyte number per surface area (e.g. Leyden, McGinley & Kligman, 1975), although no evidence is given for the advantage of this over crude counts.

3. The microbiology of dandruff

Despite the differences between the various studies there is general agreement that the main organisms found are aerobic bacteria, the acne bacillus and various pityrospora (Reddish, 1952;

Vanderwyk & Roia, 1964; Roberts, 1969; Roia & Vanderwyk, 1969; McGinley *et al.*, 1975; Leyden *et al.*, 1976; Priestley & Savin, 1976; Leyden & Kligman, 1979 and others). Of these the most constant relationship is to *Pityrosporum ovale*, and the more severe the scaling the greater the number of organisms. The relationship of other pityrospora is not clear; nor is the relationship to surface or follicular organisms and filamentous and free forms. Thus in a field of surprising inconsistency the relationship of dandruff to the pityrosporum has stood firm for many years (e.g. see Reddish, 1952).

4. Pityrosporum ovale: primary or secondary?

There are many studies of different design. Most attempt to answer the question by use of antibiotics and most are open studies of few patients. Nevertheless it is difficult to escape the conclusion that pityrosporum is the immediate cause of the scaliness and not vice versa. The only data which have been used to support a contrary conclusion are those of Kligman's group (Ackerman & Kligman, 1969; Plewig & Kligman, 1969, 1970; Marples, Downing & Kligman, 1972; Kligman *et al.*, 1974; Leyden *et al.*, 1975; McGinley *et al.*, 1975; Leyden *et al.*, 1976; Kligman *et al.*, 1979; Leyden & Kligman, 1979) and to some extent Imokawa *et al.* (1981) which will be discussed below. In general a decrease in dandruff (assessed clinically or measured as scale) and *P.ovale* has been found after selenium sulphide (Leyden *et al.*, 1976; Leyden & Kligman, 1979), zinc pyrithione (Imokawa *et al.*, 1981), amphotericin (Barber, 1977), an undecylinic acid derivative (Alexander, 1967), nystatin (Vanderwyk & Roia, 1964; Vanderwyk & Hechemy, 1967; Gosse & Vanderwyk, 1969) and econozole (Aron-Brunetiere, Dompmartin-Pernot & Droubet, 1977). The only common feature of this wide range of chemicals is their anti-fungal action. Anti-microbial agents appear to be ineffective (Leyden *et al.*, 1976; Leyden & Kligman, 1979), but the possibility of a minor role for bacteria is not resolved (see Roia & Vanderwyk, 1969; McGinley *et al.*, 1975; Vanderwyk & Hechemy, 1967). This fundamental observation of the effect of anti-fungal agents has been made by a variety of studies by different observers using so many different protocols that on general grounds one has to suspect that any error lies in the few contrary findings on which the hyperproliferative hypothesis is based. The problem which a reviewer has to face is how such a contrary view has gained the widest acceptibility despite the narrowness of its base. I find it difficult to escape the conclusion that this has more to do with communication of ideas than with their intrinsic merit.

In re-examining these contrary findings of Kligman and co-workers, a number of possible explanations become apparent. The experimental design was poor in most respects: small groups were studied and uncompensated by blindness control; the statistical treatment is rudimentary (e.g. Leyden *et al.*, 1976) with small groups analysed by group means rather than sequential analysis; use of a corneocyte counting method which was subsequently admitted (Kligman *et al.*, 1979) not to correlate with clinical appearance. Nevertheless it seems unlikely that these defects could be other than contributory, because by using them the authors (e.g. Leyden *et al.*, 1976) are able to show a decrease both in *P.ovale* and dandruff with selenium sulphide (Kligman *et al.*, 1979) and zinc pyrithione shampoos (Ackerman & Kligman, 1969; Leyden *et al.*, 1976; Leyden & Kligman, 1979). The real basis of their conclusion that *P.ovale* is not the cause is in fact their failure to show a decrease in dandruff when *P.ovale* is decreased by topical amphotericin (Leyden *et al.*, 1976; Kligman *et al.*, 1979). Their failure with nystatin in a few patients is too slight and uncontrolled to stand against the response to nystatin which others have shown in good quantitative studies. Rejection of the *P.ovale* hypothesis on the basis of this single piece of negative evidence in the face of all the other positive evidence is unsatisfactory. In the light of all the other studies with anti-fungal agents a more probable influence is that the preparation used did not achieve sufficient anti-fungal activity where it mattered. In this respect a decrease in surface organisms may of course be only distantly related to what is happening to infection in the pilosebaceous duct. In a careful re-examination of this problem by quantitative methods and using a double-blind protocol comparing amphotericin with Head & Shoulders® shampoo and a placebo (twenty-two patients in each group), there was an obvious clinical and measurable response of dandruff and viable *P.ovale* count (measured by fire-fly luciferase) to both the amphotericin application and the shampoo (Barber, 1977). It is unfortunate that this excellent study has not been published in full, although a summary is available through Procter & Gamble.

(b) The half-scalp experiments. Although few in number and with a number of technical shortcomings because they are self-controlled, dandruff improving more on the anti-fungal treated side of the scalp (e.g. with nystatin), these studies provide good supporting evidence for the role of *P.ovale* (Vanderwyk & Hechemy, 1967). The objection which has been made to this type of experiment (Kligman *et al.*, 1974) is the risk of translocation of active agents and this objection has been widely quoted. But translocation is of course irrelevant as it could only explain the *absence* of a difference. The argument is back to front: the fact that a difference occurred should be taken as practical evidence against significant translocation.

(c) The experiments of Gosse and Van der Wyke (1969) are particularly important because they show that despite continued use of the anti-fungal agent nystatin, experimental reinfection of the scalp by nystatin-resistant *P.ovale* induces relapse of dandruff in patients who have already responded to nystatin. This beautifully conceived experiment has not been given the attention it merits. It shows with classical simplicity that it is the presence or absence of fungus alone that is critical and is one of the clearest rejections of the suggestion that anti-dandruff agents act by a cytostatic mechanism.

(d) The experiments of Imokawa et al. (1981) give recent independent evidence of the relationship of *P.ovale* to dandruff and the effect of zinc pyrithione. Unfortunately they are poor experiments confounded by poor interpretation, but because they could confuse the picture which emerges from other evidence they must be considered in detail. Corneal counting was done by the method of Leyden *et al.* (1976) which has poor reproducibility (see above), some of the groups are small, the design was open and the statistical treatment dubious. Whilst in general they seem to associate *P.ovale* with dandruff, they have some reservations which arise both from defects in the study and to misinterpretation of the dissociation between organism and scale count during and after treatment: (i) The recurrence of *P.ovale* before scale when treatment is stopped for a week but *with continued non-medicated shampooing*, is a not unexpected (e.g. see Vanderwyk & Roia, 1964) lag in the time of scale formation and does not in any way argue against a pathogenic role of *P.ovale* (interestingly, their Figure 3b could well conceal an increase in corneocyte count between weeks 3 and 4). (ii) The increase in scale without an increase in *P.ovale when all shampooing (medicated and non-medicated) was stopped for 6 days* likewise does not argue against the *P.ovale* aetiology. In the absence of *all* shampooing, the anti-fungal agent (zinc pyrithione) will be left on the hair and scalp and *P.ovale* counts will therefore continue to remain low, unlike in (i) where non-medicated shampooing would have washed out the pyrithione. The observed increase in scale simply represents its greater accumulation in an unshampooed scalp. The essential but omitted control was the quantity of scale the same individuals formed when left for a comparable time without shampooing before

treatment. (iii) Finally, the evidence on which the previous argument is based is obtained by the curious and inadmissable treatment of the data in Figure 4b. The value for day 0, the baseline for this study, is comprised of a mean of ten points, all others of only six, and the significance of the results is due entirely to comparison with this baseline reading. This procedure is totally unacceptable and only the same six individuals that are followed through days 3–7 should have been used for the baseline reading in day 0.

Thus the reservations of Imokawa *et al.* (1981) are not acceptable and in fact their findings, poor though they are, of an association of *P. ovale* and dandruff and a decrease with pyrithione fit well with the *P. ovale* hypothesis.

There are few animal studies. Reddish (1952) describes studies by Durfec and Cousins (1936) of the production of infectious dandruff by topical infection of rabbit skin with *P. ovale* and its cure by topical antiseptic although the response to the dead organisms has features of psoriasis in the rabbit (Rosenberg, Belew & Bale, 1980), and Troller (1971) describes a 'dandruff-like' reaction to *P. ovale* in guinea-pigs.

Therefore my conclusions from this part of the literature are: (1) *P. ovale* is the commonest organism quantitatively associated with dandruff; (2) a decrease in *P. ovale* by a variety of anti-fungal agents decreases both the clinical appearance of dandruff and objectively measured scaling; (3) recolonization with the organism leads to recurrence of the dandruff; (4) these findings clearly indicate that *P. ovale* is the cause and not the consequence of dandruff.

5. *Kinetic studies*

The suggestion that the mode of action of various anti-fungal agents on dandruff is cytostatic and not due to their action on *P. ovale* was proposed by Kligman's group when they failed to confirm the improvement in dandruff with topical amphotericin (Leyden *et al.*, 1976; Kligman *et al.*, (1979), although they (like others) found improvement with selenium sulphide and zinc pyrithione. With the major exception of Imokawa *et al.* (1981), other authors who give support to this view do so in the main merely by repeating it without new evidence. Yet none of the papers which promote a cytostatic mechanism provides satisfactory evidence that the anti-fungal agents act by virtue of suppression of epidermal cell replication.

(a) Is there an increased epidermal cell turnover in dandruff? Few subjects have been studied, mostly by the measurement of mitotic index or percentage of basal cells taking up labelled thymidine (Plewig & Kligman, 1969; Leyden & Kligman, 1979) both of which are imprecise methods. Nevertheless the results do suggest an increased rate of cell production. However, the changes are small and if a correction were made for focal parakeratosis (Kligman *et al.*, 1979), it is not clear that the mitotic change would be found in the skin *between* the focal parakeratosis as might be expected of a 'primary disorder of hyperproliferation' (Leyden & Kligman, 1979). Thus whilst it seems likely that cell turnover will be found to be increased in dandruff its presence and degree needs to be established by reliable cytokinetic techniques in relationship to severity and in the parakeratotic scaly areas and the non-parakeratotic skin between. Finally, the aetiological importance of the alleged defect can only be shown by demonstrating its specificity by comparison with other scaly dermatoses of the scalp. I have no doubt it will fail such a test: the evidence from psoriasis, pityriasis rubra pilaris and other hyperproliferative dermatoses already tells us of that failure.

(b) Is an increased cell turnover primary or secondary? There is no evidence which establishes increased cell turnover as the primary defect in dandruff. Nor is there essential evidence that each and all of the chemically different anti-fungals which are effective in dandruff have a

cytostatic action when used *in vivo on normal skin in concentrations which are effective in dandruff*. Thus the demonstration that one anti-fungal agent decreases mitotic activity in scalp skin affected by dandruff is inadequate as this could simply be secondary to eradication of the *P. ovale* infection. As Plewig and Kligman (1969) themselves point out, studies have to be done on epidermis 'whose turnover is normal to begin with'. Unfortunately they failed to do this and the labelling index in the 'control' scalps they studied was 10%—a figure indicating an appreciable increase in mitotic activity and comparable to that which they previously found in patients with dandruff. The explanation of this finding is that their normals had the scaling of mild dandruff with an increase in corneocyte counts. This is not surprising. The scalps of most normal adult males harbour *P. ovale* (see above); many have scaling indistinguishable from dandruff except by quantity (e.g. Van Abbe, 1964; Leyden & Kligman, 1979), and Plewig and Kligman admit they found it difficult to collect individuals without obvious clinical dandruff in their prison population: it appears that they did not succeed. This study of Plewig & Kligman (1969), which is so often quoted and so little discussed, can also be dismissed because of its poor design, incompleteness and minimal numbers, with an immense measurement variation in corneocyte count given by example but not in detail so that Figure 1 is totally beyond interpretation and Figure 2 is suspect.

Because of the ubiquity of *P. ovale* and dandruff it would be difficult to do creditable experiments even on apparently normal scalp. This is apparent from the change in corneocyte counts after selenium sulphide or zinc pyrithione in 'non-dandruff subjects' (Leyden *et al.*, 1975). Thus to establish that the effect of anti-dandruff agents is primarily on cell division and not in the increased cell division occasioned by *P. ovale* infection, the studies could only be done on the very few scalps shown not to be colonized by *P. ovale* (or after eradication by a demonstrably non-cytostatic agent), or on normal skin away from the scalp e.g. the hairy skin of the forearm. In the absence of such studies the case for a cytostatic action of anti-dandruff agents is unproved and it is reasonable to assume that the effect of a range of unrelated chemicals on dandruff is by virtue of the fungicidal action they have in common. I have reviewed several *in vitro* studies (e.g. Priestley & Brown, 1980) and *in vivo* animal studies on pyrithiones (e.g. Gloor, Mildenberger & Miltenberger, 1979) which do not relate to their usage in shampoos in man, and toxicological studies showing lack of effect on prolonged application in mouse skin (e.g. Procter & Gamble internal reports). Thus the allegation that 'Most . . . agents which are beneficial in dandruff exert cytostatic effects' (Parekh, Min & Goldberg, 1970) is without foundation.

It should be noted (i) that there has been no systematic study of the effect of undisputed cytostatic drugs on dandruff; most of the work has been by the curiously perverse approach of attempting to show that a few selected anti-fungal agents are cytostatic; (ii) that even if cytostatic drugs were active against dandruff this would not refute a primary role of a fungal infection since they might simply block one of the consequences of the fungal action; (iii) there is clinical evidence that cytostatic agents may worsen fungal infections although their appearance may be modified. Thus it would be disadvantageous to look for new anti-fungal agents by screening for cytostatic activity. In this respect the recommendation of the U.S. Advisory Review Panel on OTC preparations (1980) on the use of cytostatic agents for the control of dandruff is as remarkable as it is unacceptable.

(c) Theoretical considerations. '. . . dandruff is a disorder of hyperproliferation' (Leyden & Kligman, 1979). The absurdity of the cytostatic argument is best perceived by allowing it. Thus it immediately becomes necessary to explain a non-causally related association of fungus and dandruff as well as an independent reason for the increased cell turnover. If, as the cytostaticists

imply, the increased turnover is primary and since it is apparent on the head and not the tail, we arrive at a definition of dandruff as 'a primary hyperproliferative disease of the scalp with a specific susceptibility to asymptomatic pityrosporum infection'. It is clear that the intellectual currency necessary to set up house with the cytostatic theory would leave no credit for the never-ending line of dependent consequences.

6. *The mode of action of anti-fungal agents in dandruff*

There are many different chemical agents which ameliorate dandruff and their only common effect is that on *P. ovale* and similar fungi. Their precise mode of action may well differ: pyrithione, for example, has effects on membrane transport and on other aspects of cell structure and macromolecular synthesis and function (Chandler & Segel, 1978).

7. *Miscellaneous*

There are a number of poorly defined investigations, e.g. the relationship of the fungal action to sebum and sebum products should be resolved; the negative studies of Kligman *et al.* (1979) are particularly unconvincing. The relevance of work on fatty acids (Marples *et al.*, 1972) is not clear and the purpose of studying the effect of pityrosporum extracts on the rabbit ear (which has been given the pre-emptive anthropomorphism 'comedogenic') (Weary, 1970) would be more clear if only blackheads occurred in the rabbit scalp. Likewise, studies of the effect of intradermal injection of fungal extracts, the role of the immune system, complement activation etc., would be easier to understand had they been more sharply pointed at answerable questions about host factors and response.

Remaining questions

The questions which remain to be answered are: (i) how does the infection lead to the scaling? The fashionable possibilities list themselves. (ii) What is the basis of individual susceptibility to *P. ovale* infection and dandruff? (iii) What is the role of this infection in other diseases? How much of seborrhoeic eczema results from colonization by pityrosporum and how much even of scalp psoriasis is a pityrosporal Koebner phenomenon? The advent of new potent anti-fungals such as ketaconazole will allow us the luxury of a short-cut to the answer.

Conclusions

The clinical and histopathological dissociation of dandruff from early seborrhoeic eczema and psoriasis has not been made. *P. ovale* is the commonest associate of dandruff: remove it and the dandruff improves, recolonize and the dandruff recurs. How *P. ovale* induces dandruff is not clear; inflammation and desquamation with some increase in cell production is an end result. There is no evidence that anti-fungal agents act on dandruff other than by decreasing the number or activity of the fungal population and the few experiments alleging a primary anti-mitotic effect are unacceptable. It is alarming that the literature is so curiously biased in favour of a view which is clearly at odds with most work in the field as well as being tedious conceptually.

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Chronic actinic dermatitis

SIR, Dr Diffey and colleagues (*British Journal of Dermatology* 1984, **110**, 190) in their paper on a dermatological photobiology service, suggest that the term 'chronic actinic dermatitis' was introduced by Ramsay in 1981. I think it should be noted that this term was first proposed by Hawk and Magnus (1979) to encompass both actinic reticuloid and photosensitive eczema and transitional states between them.

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Lasers, psoriasis and the public

SIR, We may be in the age of the laser but it is important to understand the limitations of these tools. There are some claims which have sprung up in medicine and cosmetology which test the credulity of the most open-minded. Advertisements have appeared in the lay press claiming 'effective elimination of wrinkles', 'elimination of lines . . . and all skin problems', and a laser which 'takes years off your face'. Four examples appeared in the Advertising Standards Authority Case Reports in January 1983 in which complaints against an advertisement were upheld.

We have been in contact with the Psoriasis Association because members wished to know the truth of these and other verbal assurances of the beneficial effect of lasers in psoriasis. There are no reports in the literature of psoriatic skin benefiting from laser light. For the last year, we have been working with the bioLas D helium–neon laser. It emits at 632 8 nm with a power of 5 mW. At 15 cm the power density is 0·33 mW per cm^2. The effective penetration depth is said to be 1·5 cm from a distance of 15 cm. It is a continuous wave monofibre instrument of the class 3A. Previous work with the helium–neon laser has shown accelerated healing of experimentally inflicted wounds (Averbakh, Sorkin & Dobkin, 1976), and an enhanced rate of wound closure in rats (Kana *et al.*, 1981), but venous ulcer healing was not improved (Monfrecola, Martellotta & Lembo, 1982). The effect on cell division seems to depend on a narrow power band and the nature of the tissue. In the neonatal chick, low-dose radiation inhibits cell division in the lens (Poon & Yew, 1980) but increases the number of mitoses in the retinal pigment epithelial cell (Yew, Lam & Chan, 1982). Our work has shown an inhibitory effect on angiogenesis in the chick chorioallantoic membrane. We also investigated the effect on plaques of psoriasis. Ten plaques on seven patients were irradiated from a distance of 15 cm for 30 minutes each day for 7 days. The treated areas behaved no differently from contiguous or symmetrically distributed plaques during the follow-up time of 3 months.

One might expect an inhibitor of angiogenesis to affect the course of psoriasis. Development of the Kobner phenomenon (the occurrence of a disease at the site of damaged skin, in this case psoriasis) supports the view that angiogenesis is associated with the psoriatic process. Ryan (1976) demonstrated this by showing that while the caps of ointment tubes pressed on the skin elicited the Kobner phenomenon, no psoriasis developed where the indentation marks had obliterated the vessels. The reaction is also inhibited by vasoconstriction (Telner & Fekete, 1961).

Although lasers are remarkable, they are only lamps. Some therapeutic effects have been found. Meanwhile, people are being exposed to certain lasers whose effects may be non-existent, placebo or even hazardous.

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Lymphocyte subsets in pagetoid reticulosis

SIR, Mackie & Turbitt show convincingly that their case of pagetoid reticulosis was characterized by a preponderance of T8 (cytotoxic/suppressor) epidermal lymphocytes (*British Journal of Dermatology* 1984, **110**, 89–94). However, their conclusion that pagetoid reticulosis is distinct from mycosis fungoides may not be fully justified in all instances. We too have evaluated a case of pagetoid reticulosis using monoclonal antibodies.

A female aged 80 years presented with an expanding plaque on the dorsum of the left wrist. The lesion had been present for 18 months. Light microscopy showed acanthotic hyperkeratotic epidermis and an epidermotrophic cellular infiltrate (Fig. 1a). A dual population of small lymphocytes and larger cells with pale nuclei were present (Fig. 1b). The latter showed no convincing ultrastructural evidence of histiocytic differentiation. The clinical and histological diagnosis was considered typical of pagetoid reticulosis.

Immunoperoxidase investigations were performed on frozen sections and paraffin-embedded tissue. The monoclonal antibodies were used at a titre of 1 in 400 with appropriate controls to confirm the specificity of the technique. All small lymphocytes bore T11 (pan T) antigen but, in marked contrast to the findings of Mackie & Turbitt, the principal infiltrating epidermal lymphocyte was of T4 (helper/inducer) subset (Fig. 1c). Few T8 (cytotoxic/suppressor) lymphocytes were present within the epidermis but the dermal T4:T8 ratio was 2:1 (Fig. 1d). The larger pale infiltrating epidermal cells were also of helper phenotype (T4 positive; T8 negative). Only occasional small histiocytes were identified using Mo2, lysozyme and α_1-antitrypsin antibodies. No T10 (immature and activated lymphocyte phenotype) or Leu 7 (natural killer) cells were present. T6 dendritic (Langerhans) cells were focally reduced within the epidermis but present within the dermis in small numbers.

These observations are a complete mirror image of those reported by Mackie & Turbitt. Our results indicate that pagetoid reticulosis can also be characterized by a preponderance of T4 (helper/inducer) epidermal lymphocytes. Phenotypically, these findings bear close similarity to those reported previously in mycosis fungoides and Sézary syndrome. No absolute evidence was obtained to define the lineage of the larger pale epidermal cells. Although negative for standard histiocyte and dendritic cell markers, T4 antigen can be expressed on both lymphocytes and cells of the monocyte–phagocytic system.

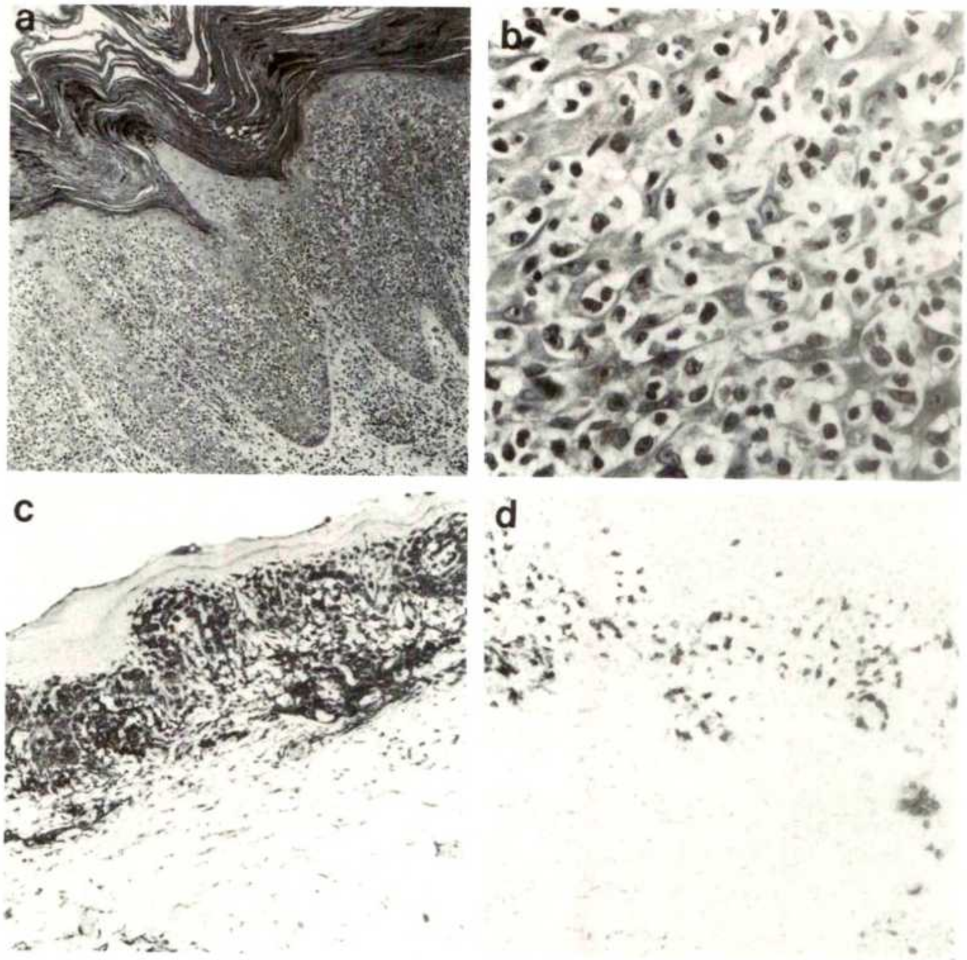

FIGURE 1. (a) Acanthotic hyperkeratotic epidermis with an epidermotrophic pagetoid cellular infiltrate (haematoxylin and eosin, × 20). (b) Epidermis showing pagetoid infiltration by a dual population of cells (H & E, × 360). (c) T4 immunoperoxidase showing intense staining of infiltrating epidermal cells and also dermal lymphocytes (× 40). (d) T8 immunoperoxidase showing plentiful dermal, but few epidermal, lymphocytes (× 40).

It appears that pagetoid reticulosis may represent an immunologically heterogeneous group of cutaneous diseases which possess a similar clinical and histological appearance. However, it is possible that different subgroups may have differing prognostic implications. The subject is made even more complicated by the recent description of a verrucoid epidermotrophic OKT8-positive lymphoma, although this did not display the clinical appearance of pagetoid reticulosis (Caputo *et al.*, 1983). Clearly, further cases of pagetoid reticulosis must be studied before definitive conclusions can be drawn.

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The treatment of steatocystoma multiplex suppurativum with isotretinoin

Sir, Steatocystoma multiplex suppurativum (SMS) is an uncommon disorder in which numerous cystic lesions appear across the trunk and limbs, commencing in adolescence. On occasions individual lesions become severely inflamed and suppurate and in this stage SMS may resemble cystic acne.

We have seen three male patients aged 22, 25 and 28 years with SMS over the past 2 years; they had no family history of SMS but presented as 'refractory cases of cystic acne' with very extensive cystic lesions and continued suppuration of numerous truncal cysts. They had each received prolonged courses of tetracycline and erythromycin in doses of 250 mg four times daily with minimal improvement. Isotretinoin was administered in a dosage of 1 mg per kg per day to each patient for a period of 6 months (after informed consent). Fasting lipid levels and liver function tests were performed prior to starting therapy and at monthly intervals throughout therapy.

Within 1 month of commencing isotretinoin all patients showed an improvement which continued throughout the treatment period to the extent that there was a virtual elimination of inflamed and suppurating lesions at the end of treatment. There was no apparent effect on the non-inflamed cystic lesions. After stopping treatment the patients have been observed for a further 3 to 8 months during which the inflammatory activity has remained minimal.

Apart from cheilitis and facial dermatitis no other side-effects were encountered; lipid profiles and liver functions tests remained within normal limits throughout therapy.

The aetiology of the inflammatory lesions in SMS is unclear but there would appear to be two possible explanations: either primary bacterial infection of the cystic lesions, or alternatively rupture of the cyst wall with leakage of their contents into the dermis.

Isotretinoin has no direct antibacterial effect (King *et al.*, 1983) but it is known to possess anti-inflammatory properties (Pigatto *et al.*, 1983). A mechanism involving a reduction in the sebum excretion rate would seem unlikely because although the cystic lesions are known to contain unsplit esters of sebum (Nicolaides & Wells, 1957) the isotretinoin produced no apparent reduction in the size or number of non-inflamed cystic lesions. A direct action of isotretinoin on the epidermal component of the cyst wall cannot be ruled out.

From our experience in these three cases we would recommend isotretinoin as the treatment of choice for cases of SMS with a severe inflammatory component.

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Autoantibodies in alopecia areata

SIR, The paper by Galbraith *et al.* (*British Journal of Dermatology* 1984, **110,** 163) prompts us to report our surprisingly different data on non-organ-specific autoantibodies in alopecia areata.

The authors were able to detect only one antinuclear antibody out of sixty cases, when testing their sera diluted 1:10. It should be stressed that, when testing sera at 1:10 dilution, a variable proportion (7–13% in our experience depending on the autoantibody considered) of healthy subjects are also positive, at least for antinuclear (ANA) and anti-smooth muscle (SMA) antibodies. In fact, as far as liver diseases are concerned, there is a general agreement that sera should be tested at $\geqslant$1:20 dilutions to obtain reliable results (Mackay, 1983). When we tested eighty-six sera from alopecia cases at 1:10 dilution, non-organ-specific autoantibodies (namely ANA, SMA, anti-mitochondrial and anti-basal cell layer antibodies) were found (single or associated) in fifty-five (64%). SMA was the most prevalent. Further dilution (1:40) of sera lowered the prevalence to 41%, which was still significantly elevated, when compared with that of fifty healthy subjects (0%). No difference was detected as to the age and sex distribution, extent (patches, totalis or universalis) and duration of the disease and topical treatment with contact agents.

The reasons for this discrepancy are not immediately clear. The median age of our patients was similar to that of theirs: 27·5 years (range 6–74 years) and, so far as we can tell, there seem to be no major technical differences in the fluorescence test (except for the incubation at 4°C, which certainly reduces reactivity of the antibodies). Geographical variations are possibly involved as well. Nevertheless, preliminary results obtained by testing organ-specific autoantibodies (anti-human thyroid and gastric microsomes) seem to be in agreement with figures reported by Galbraith *et al.* (1984). In fact our overall prevalence is 38%.

In conclusion, alopecia areata is certainly characterized by significant immunological abnormalities, but conflicting results are still being reported, which are difficult to understand.

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Retinoids and PUVA in psoriasis

SIR, We are surprised that Parker *et al.* (*British Journal of Dermatology* 1984, **110,** 215) found no advantage in combining etretinate with PUVA. Our results originally published in 1978 (Fritsch *et al.*, 1978a) have not only been reproduced by ourselves (Fritsch *et al.*, 1978b; Wolff & Hönigsmann, 1981) but also by others (Orfanos, Pullmann & Enderer, 1978; Heidbreder & Christophers, 1979; Frenk, 1980; Lauharanta, Juvakoski & Lassus, 1980; Grupper & Berretti, 1981), and we are therefore at a loss to explain what went wrong with the Newcastle study. The procedural differences mentioned by Parker *et al.* may be one explanation but certainly not the most satisfactory one. Admittedly, our own studies were not double-blind placebo controlled but this would have been both unnecessary and meaningless: in our original reports, (Fritsch *et al.*, 1978a,b) the improvement of PUVA–etretinate over PUVA alone was 30% for duration of treatment and 50% for reduction of UV-A radiation load which, since we were dealing with severe, widespread psoriasis, was certainly far in excess of what could be attributed to a placebo effect even by firm believers in placebo treatment of psoriasis. More importantly, etretinate produces dryness of lips and cheilitis, noticeable both to the patient and physician within a few days after initiation of therapy which makes any attempt to run a blind study with this drug a difficult task. We are indeed curious to know how the placebo-controlled regimen was carried out in a blind fashion in Newcastle when thirteen of fifteen of their patients taking etretinate developed cheilitis and a fifth had hair loss.

In the final analysis of the Parker results only fourteen patients on PUVA–etretinate and nine patients on PUVA–placebo were left to be evaluated. We question whether statistical tests can yield meaningful results in such small numbers of subjects. Since the clearance data in the Parker study are at variance with all other large-scale studies on PUVA plus retinoids [for instance, 40 days in the Parker study v. 23 days in our study (Hönigsmann & Wolff, 1983)], they should be confirmed by larger number of patients. The shortcomings in interpreting data obtained with only a few patients are quite evident when one considers that four out of thirteen patients (31%) in the PUVA–placebo group failed to clear. It is hard for us to believe that the PUVA failure rate is indeed as high as that in Newcastle. Also, Parker *et al.* suggest that in our studies patients not on retinoids may have been treated with more PUVA than was necessary; this is unlikely because our average total UV-A doses were far less than in other large-scale studies.

Published evidence from most centres clearly shows that retinoids represent a major breakthrough in combined psoriasis therapy, and it is highly unlikely that all but the group in Newcastle were wrong. PUVA–etretinate is therefore an established treatment for severe psoriasis in Vienna and elsewhere, and since etretinate is given only for short periods of time its use is only limited by concerns of teratogenicity. Similar beneficial results have been obtained with a combination of isotretinoin (13-*cis* retinoic acid) and PUVA (Hönigsmann & Wolff, 1983). Since 13-*cis* retinoic acid is cleared much faster from the body, we consider its use a welcome alternative in the combined treatment of women of child-bearing age.

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British Journal of Dermatology (1984) **111**, 249–251.

Book Reviews

Allergic Contact Dermatitis to Ingredients used in Topically Applied Pharmaceutical Products and Cosmetics. A.DOOMS-GOOSSENS (1983) Leuven: Leuven University. Pp. 164. Price BF 57.10.

This small book really consists of three separate sections.

The first deals with the epidemiological aspects of allergic contact dermatitis and the use that can be made of a computer-assisted monitoring system in identifying allergens in the environment and in assisting patients with known sensitivities. This section is really essential reading for anyone who might be thinking of putting their patch test records on a computer.

The second component is a very learned thesis on the impurities in petrolatums. It illustrates many of the sophisticated physico-chemical techniques that can now be employed in the investigation of cases of allergic contact dermatitis.

The third, and to my mind the most important part of this book, deals with the influence of 'external factors' on the allergenicity of topically applied pharmaceutical products and cosmetics. This includes the influence of 'source', processing methods, degradation and the interactions that may occur between compounds during storage. There is also an extremely lucid discussion on the phenomenon of compound allergy and 'quenching'. Another contentious subject which is looked at in some detail is the problem of 'hypoallergenicity' which is approached from a pharmaceutical viewpoint, looking at ways for rendering products less sensitizing, and the role of ingredient labelling and allergen avoidance. Many other important topics are also considered including the problems of cross-sensitivity, group sensitization and the legislative aspects of allergic contact dermatitis.

This is an excellent book, and one that I can wholeheartedly recommend to all dermatologists, not just those specifically involved in contact dermatitis. It is extremely well written, well referenced and full of useful information.

J.D.WILKINSON

The Year Book of Dermatology. Edited by A.J.SOBER AND T.B.FITZPATRICK (1983) Chicago: Year Book Medical Publishers Inc. Price £32.00.

The 1983 *Year Book of Dermatology* is the equal or peer of its predecessors. With extracts from eighty-four journals even the most avid bibliophile will have his knowledge augmented, while the careful selection of articles will lessen the despair felt by the average reader in his conviction that too few new facts are being chased by too many authors.

For 1983 the Editorship has moved north from South Carolina to Harvard, and in Professors A.J.Sober and T.B.Fitzpatrick, the change in editorial accent is reflected in a regrouping and expansion of the chapter headings. The customary initial and long review article is omitted and there must be immediate and future appeal in the list of some thirty references to pertinent reviews published during 1982. The format is otherwise as before, with admirable summaries followed by perceptive comments from the Editors and their dozen Contributory Editors. New treatments, e.g. synthetic retinoids, are dealt with fully, while the changing nature of diagnosis and disease is fully annotated. In the past, disease has been capricious with spontaneous mutations and permutations, but at the end of the 20th century some of these changes occur in the wake of the hosts' permissive and changed social habits. Acyclovir is a potential support line for the herpes sufferer, but no such solace is available in the Acquired Immune Deficiency Syndrome. Its devastation is reaped not only by those who sow, but by such innocent bystanders as haemophiliacs and the recipients of blood transfusion. A.I.D.S. is covered in six reviews and is of such importance that in future the Editors may consider making the references less elusive in the index.

The lineage of articles reviewed is of interest. My initial impression and surprise that the relatively new

Journal of the American Academy of Dermatology had outstripped all others was confirmed by my count of forty-three articles extracted. The A.M.A. *Archives of Dermatology* provided thirty-five articles. Ill-suppressed jingoism was allayed by my counts of thirty-three and fourteen reviews from the *British Journal of Dermatology* and *Clinical and Experimental Dermatology* respectively. These figures, together with the twenty-nine articles culled from *Acta Dermato-venereologica*, must be a source of satisfaction to their Editors.

An innovation is a brief section on 'Statistics of Interest to the Dermatologist'. California and New York lead the Top Ten States with a staggering 906 and 554 Dermatologists respectively. By championing female suffrage Wyoming earned itself the name of the Equality State, but is second bottom of the list with six Dermatologists. The hardy and job hungry should note that with only two Dermatologists Alaska continues to justify its alternative name of The Last Frontier.

A.W.McKENZIE

Cosmetic Dermatologic Surgery. S.J.STEGMAN AND T.A.TROMOVITCH (1983) Chicago: Year Book Medical Publishers. Price £34.50.

Much of dermatology (and plastic surgery) is cosmetic in its intentions. The really serious effects of psoriasis, acne, alopecia, hirsutes are on the patient's mind, not his body, in the great majority of cases. In the past when the struggle for survival against tuberculosis, plague, cholera, typhoid, and malnutrition dominated medicine, mere surface blemishes could be afforded little time or effort. This is still true in many poor countries today. But, in the richer countries, luxuries are possible and one of the most sought-after is a perfect skin. Even in my dermatological life-time the horrors of lupus vulgaris, advanced rodent ulcer and gross infections have been replaced in the clinics by the cosmetic dermatoses—fainter and fainter rashes alternating with smaller and smaller lesions. We have no need to be cynical about this. It is simply a reflection of our growing control of certain diseases and the higher expectations of an increasingly sophisticated society. And we can only welcome and encourage the earlier presentation of skin malignancies which permits more effective treatment.

This book is an expression of one aspect of the modern dermatologist's expertise. A good deal of overlap is evident and to be expected between his work and that of the plastic surgeon on the one hand, and the beauty parlour on the other. There are detailed descriptions of chemical face peels, dermabrasion, hair transplant and collagen implants. Some of us may feel there is too much emphasis on some of these techniques but we must remember that this volume is written for a wider public than the British-trained dermatologist.

The production of the book is excellent but some photographs are over-exposed and some of the 'before and after' type are less than convincing. There can be little doubt however of the wealth of personal experience of the authors and the book is clearly printed and well written. The discussion of methods to reduce post-acne scarring and the description of collagen implants are particularly well done. Most of the advice and descriptions seem sensible and useful. But not all of us would agree that the best treatment for xanthelasmata is excision and primary closure, that a friction blister separates epidermis from dermis, or that the best treatment for syringomas is surgical excision. And whatever happened to rhinophyma? Also the advice to employ a cardiac monitor and an I.V. line during phenol face-peels because of the presumably fairly common cardiac arrhythmias induced by such procedures would propel most British dermatologists into placidly curetting off a few more seborrhoeic warts rather than paddle their canoe in such potentially turbulent waters.

The author's emphasis on pre-operative photography and a very full discussion with the patient before embarking on any procedure, is wise and necessary counsel, geared especially to the lawyer-ridden U.S.A., but complacency in other countries about such matters could be mistaken.

The price (£34.50) seems high for a soft-backed book but anyone seriously interested in the techniques of cosmetic dermatology will want to buy it.

J.S.COMAISH

Dermatologia Vascolare. V.PETRUZZELLIS AND A.SCARDIGNO (1983) Bari Caterza. Pp. 214.

This Italian text on vascular diseases as seen in a dermatology department is elegantly laid out and nicely illustrated with eye-catching diagrams and clinical photographs, in such a way that only a limited knowledge of Italian is required. The authors work as specialists on vascular diseases in the Department of Dermatology at Bari. The book is at a level for trainee dermatologists and others interested in peripheral vascular diseases, also general practitioners. It is not intended to be a major monograph for the expert to use as a source book.

The information contained is mainly available in monographs from various specialties, but is here brought together in an attractive and useful way with a bias towards dermatology. The literature quoted is mainly from the English language, but also with references to Italian works. We are introduced to a number of drugs not currently much used by English dermatologists. Leg ulcers and vasculitis are not dealt with at any depth. There is a useful last chapter on the various investigations currently available for investigating peripheral vascular disease. R.H.CHAMPION

Current Issues in Dermatology, Volume 1. Edited by J.P.CALLEN, M.V.DAHL, L.E.GOLITZ, J.E.RASMUSSEN AND S.J.STEGMAN. Boston: G.K.Hall.

Most practising dermatologists are overwhelmed by the amount of reading material which arrives on their desks. Each year a number of new journals are produced, and selection and rejection of the excessive dermatological literature becomes inevitable. This new monograph, appearing to be the first in an annual series, has little to recommend it. It attempts to update the specialist dermatologist, yet much of the information in the text is contained in routine undergraduate teaching. We have heard that Negro children develop keloids when their ears are pierced, that mongolian spots are flat, blue-black lumbosacral lesions and we may not be interested in a list three and a half pages long containing a 'partial listing' of disorders of neutrophil function. Nor are we very interested in a scalpel with heated blade which costs £2000, or in the thoroughly advertised collagen implants which may have a big application to the wealthy lady who has an excess of money to spend on trying to make herself look young.

The text is printed as though the script and photographs are reproduced by a photocopying technique and the quality is appropriately bad, and the photomicrographs of histology are worthless. Some of the articles are well written and interesting but the publication as a whole can be rejected out of hand as an inferior copy of the already well established, useful, *Year Book of Dermatology*. D.G.MUNRO

Hair Root Characteristics of the Human Scalp in Health and Disease. J.D.R. PEEREBOOM-WYNIA (1982) M.D. Thesis, Erasmus University of Rotterdam. Pp. 122.

A quantitative study of the hair root status of the scalp can be a valuable diagnostic procedure. To obtain reliable and reproducible findings, the technique of plucking the hair must be standardized and such factors as recent shampooing or other hairdressing procedures be taken into account.

In this MD thesis, Dr Peereboom-Wynia first reviews the literature, then describes in detail the technique which she found to be best, which adds to the range of measurements that can usefully be recorded, including the hair root shape and the presence or absence of the hair root sheath, also the angulation of hair root or shaft and deformities in their contours. She then applies her technique to many normal and abnormal scalps and demonstrates convincingly that, with greater attention to detail than is customary, it is possible to derive considerably more useful information from these simple tests.

This very helpful monograph is essential reading for all who use root status studies for diagnosis or research. ARTHUR ROOK

British Journal of Dermatology (1984) **111**, 252.

News and Notices

Fellowship of the Royal College of Physicians (London)

The following Dermatologists have recently been elected F.R.C.P. (London): P.S.Friedmann, J.D.T.Kirby, P.C.H.Newbold and D.I.Porter.

Therapeutic Photomedicine, Vienna, February 1985

The 1985 Clinically Oriented Symposium of the European Society for Dermatological Research (ESDR) will be held jointly with the Arbeitsgemeinschaft Dermatologische Forschung (ADF), 20–22 February 1985 at Baden/Vienna, Austria.

The major part of this symposium consists of lectures given by invited experts, but there is also some time allotted for a limited number of free communications. The main subjects to be covered are: basic terminology, photochemistry, molecular and immunological aspects, therapeutic principles and long-term hazards of phototherapy.

For further information contact Herbert Hönigsmann, M.D., c/o Vienna Academy of Postgraduate Medical Education, Alser Strasse 4, A-1090 Vienna, Austria.

Medical Questionnaire for Alopecia Areata Patients

Help Alopecia International Research, Inc., a non-profit, research-oriented organization, has prepared an eleven-page medical questionnaire for international distribution. The questionnaire, organized with the assistance of four specialists in dermatology, psychology, psychiatry and immunology, contains extensive question sections on each of these fields of research. Help Alopecia International Research, Inc., will computerize the returned information, have it analysed and make this information available for all people involved with alopecia areata, totalis and universalis. The goal is to assist in research programmes geared to seeking a cure for this puzzling condition. All physicians wishing to participate are invited to write for questionnaires directly to: H.A.I.R., Inc., P.O. Box 691487, Los Angeles, California 90069, U.S.A.

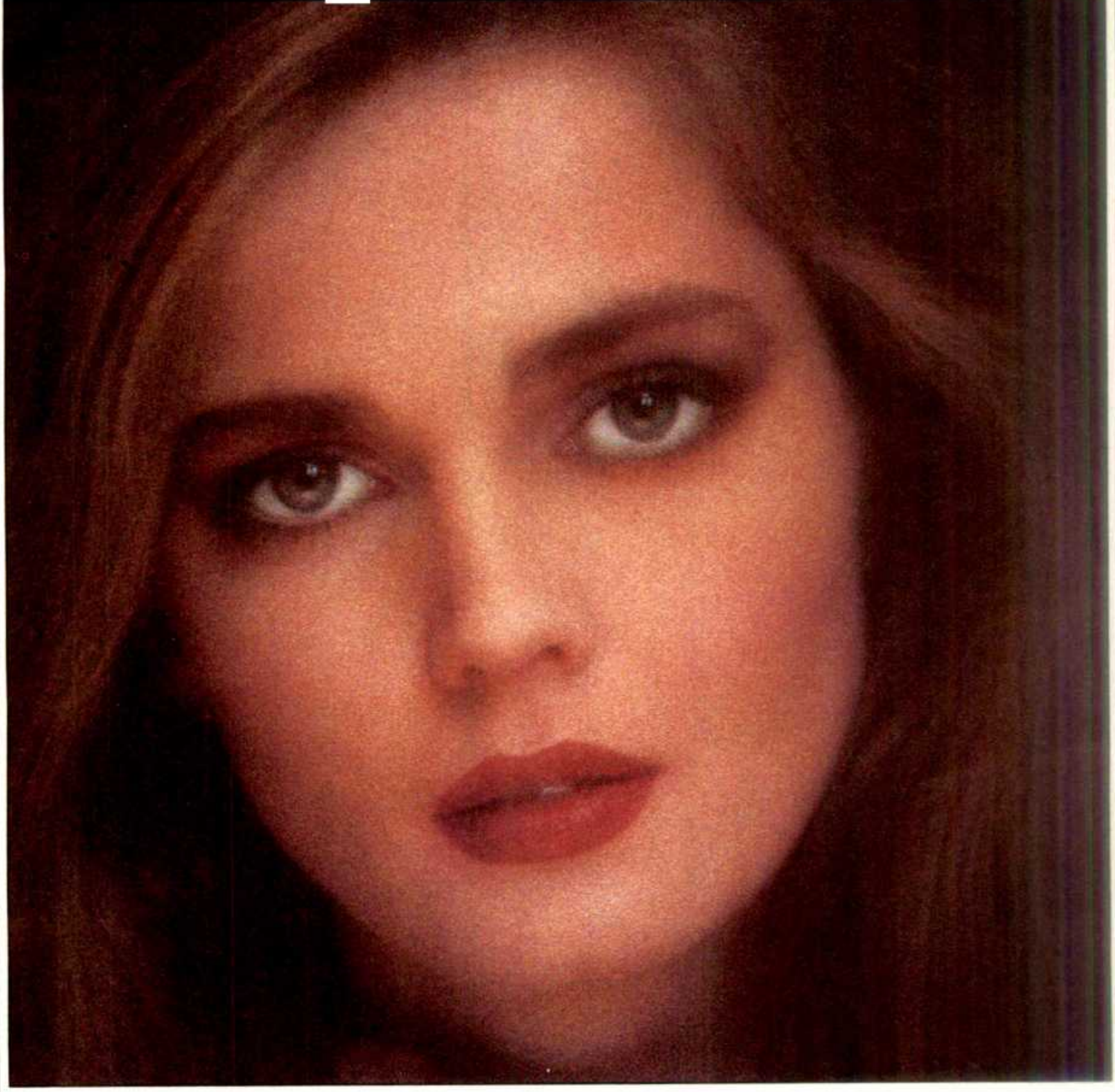
European Society of Paediatric Dermatology, Münster, October, 1984

The inaugural meeting of the European Society of Paediatric Dermatology will be held in Münster, West Germany on the 5th and 6th October 1984. For further information please contact Professor R.Happle, at Von-Esmarch-Strassen 56, 4400 Münster, F.R.G.

Introductory Course on the Biology of the Skin, Cambridge, December 1984

The annual introductory course 'The Biology of the Skin' will be held in Fitzwilliam College, Cambridge from 10 to 14 December 1984. This course is primarily for registrars and postgraduate students at an early stage of their training in dermatology but is open to other interested practitioners. Closing date for applications: 23 November 1984. Further information may be obtained from The Conference Secretary (BOS), Posgraduate Medical Centre, Clinical School, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, U.K. Tel. Cambridge (0223) 212216.

THE ACCEPTABLE FACE OF COAL TAR



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In chronic psoriasis, Alphosyl offers the effectiveness of coal tar with its direct antiproliferative action on epidermal cells, together with the benefits of allantoin which helps remove plaque scales.

Alphosyl, available as a Cream, Lotion and Shampoo (Alphosyl Application PC) is therefore the ideal maintenance therapy for most types of chronic psoriasis.

ACCEPTABLE TO PATIENTS

Unlike crude coal tar preparations, Alphosyl is easy to apply, has an unobtrusive smell, and is formulated to minimise staining.

Additionally, unlike dithranol, Alphosyl will not damage healthy skin. This combination of effectiveness and cosmetic acceptability encourages patient compliance and simplifies the management of chronic psoriatic conditions.

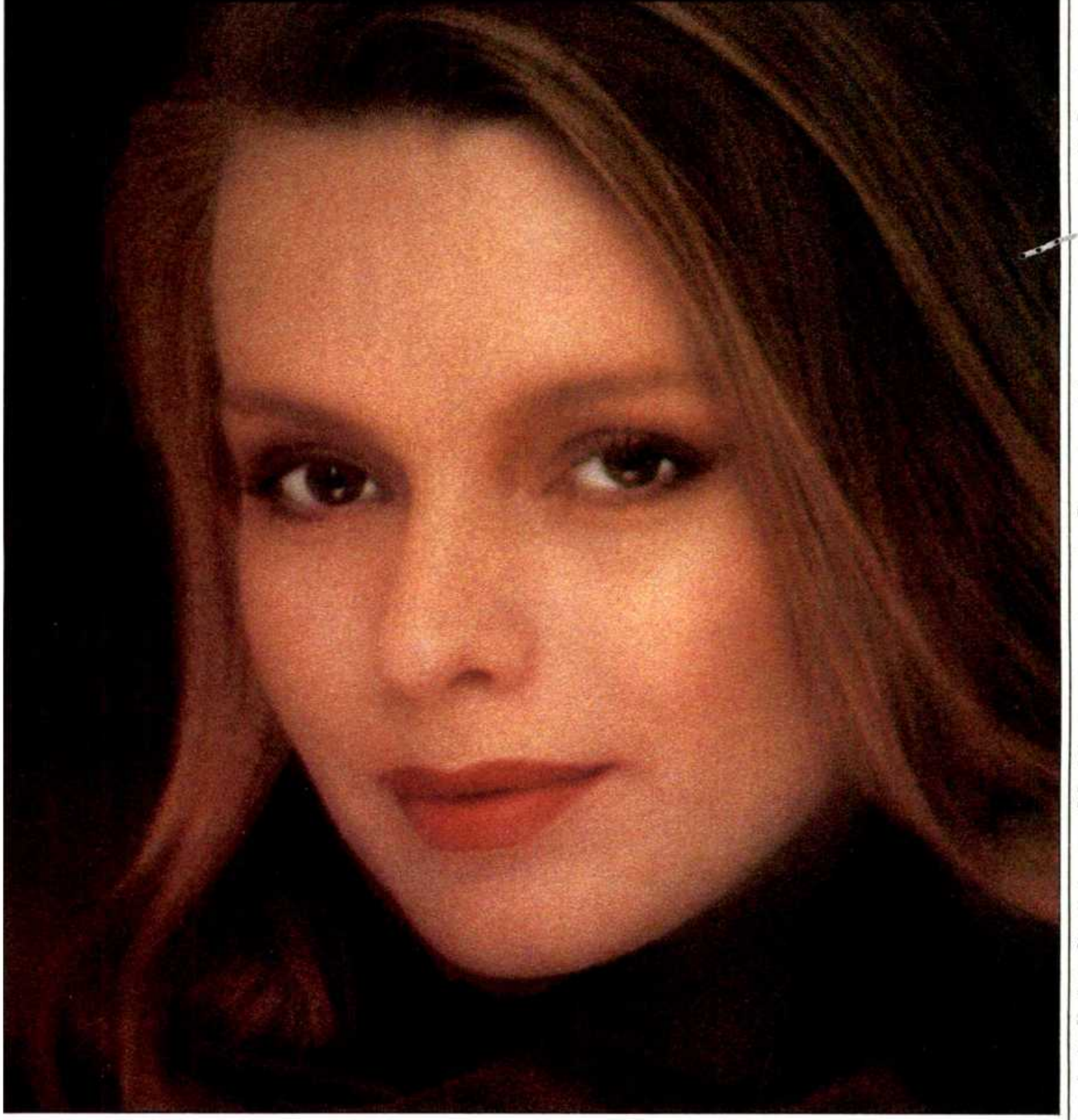
ALPHOSYL CREAM AND LOTION

(coal tar 5%, allantoin 2%)

FULLER FREEDOM FROM PSORIASIS

Presentation LOTION AND CREAM. Containing refined alcoholic extract of coal tar 5%, allantoin 2% in a greaseless vanishing-cream base. The lotion is a light free-flowing emulsion and the cream is smooth and homogeneous, light tan in colour. **Uses** Dermatological stimulant for the treatment of psoriasis. **Dosage and administration** Apply liberally two to four times daily, massage well into the affected areas. Occlusive dressings are unnecessary. **Contra-indications, warnings etc.** For external use only. Discontinue if irritation occurs or in cases of sensitivity to coal tar. **Pharmaceutical Precautions** Do not refrigerate. Store at room temperature. **Legal category** P. **Package quantities/Basic NHS Cost** ALPHOSYL LOTION. Bottle of 250ml., £2.31. ALPHOSYL CREAM. Tube of 75g., £1.59. **Further information** Alphosyl Lotion is the basic treatment for psoriasis of the body and scalp. Alphosyl Cream is designed especially for lubrication in the intertriginous areas. **Product Licence Nos.** Alphosyl Lotion 0036/5008. Alphosyl Cream 0036/5006. Further information is available on request. Stafford-Miller Ltd., Professional Relations Division, Hatfield, Herts AL10 0NZ.

THE ACCEPTABLE FACE OF STEROIDS



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Alphosyl HC contains low dose hydrocortisone, coal tar and allantoin for the treatment of mild to moderate psoriasis. It rapidly alleviates unsightly inflammation and avoids the need for potent steroids. Alphosyl HC can be used when transferring patients from more potent therapy.

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Unlike many alternative treatments, Alphosyl HC has an unobtrusive smell and is formulated in a vanishing cream base to minimise staining.

Its combination of effectiveness and cosmetic acceptability encourages patients to comply with your prescription.

ALPHOSYL HC

(coal tar 5%, allantoin 2%, hydrocortisone 0.5%)

FULLER FREEDOM FROM PSORIASIS

Presentation A cream containing refined alcoholic extract of coal tar 5%, allantoin 2% and hydrocortisone PhEur. 0.5% in a greaseless vanishing-cream base. The cream is smooth, homogeneous and is light tan in colour. **Uses** Dermatological stimulant and local corticosteroid anti-inflammatory therapy for psoriasis. **Dosage and administration** Apply two to four times daily, massaging well into the affected areas. **Contra-indications, warnings etc.** In infants long-term continuous therapy should be avoided. Adrenal suppression can occur even without occlusion. Topical administration of corticosteroids to pregnant animals can cause abnormality of foetal development. The relevance of this finding to human beings has not been established. However, topical steroids should not be used extensively in pregnancy, i.e. in large amounts or for long periods. **Pharmaceutical Precautions** Store at room temperature. Do not refrigerate. **Legal category POM. Package quantities** Tubes of 30g. and 45g. **Basic NHS cost** £1.73 for 30g., £2.46 for 45g. **Further information** Alphosyl HC cream is intended for the treatment of the acute stages of psoriasis when the basic Alphosyl action is enhanced by the addition of hydrocortisone. **Product Licence No.** 0036/0026. Further information is available on request. Stafford-Miller Ltd., Professional Relations Division, Hatfield, Herts. AL10 0NZ.

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The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Manuscripts should be sent to the Editor, Dr J.L.Burton, Dermatology Department, Bristol Royal Infirmary, Bristol BS2 8HW. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. A duplicate copy, complete with figures, tables and references, should be sent, since in many cases two referees are consulted. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3}g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hours(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations, A Guide for Biological and Medical Editors and Authors* (1977) The Royal Society of Medicine, London.

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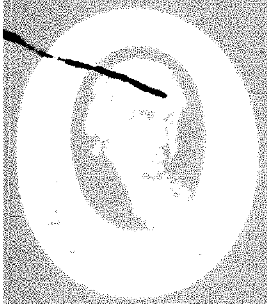

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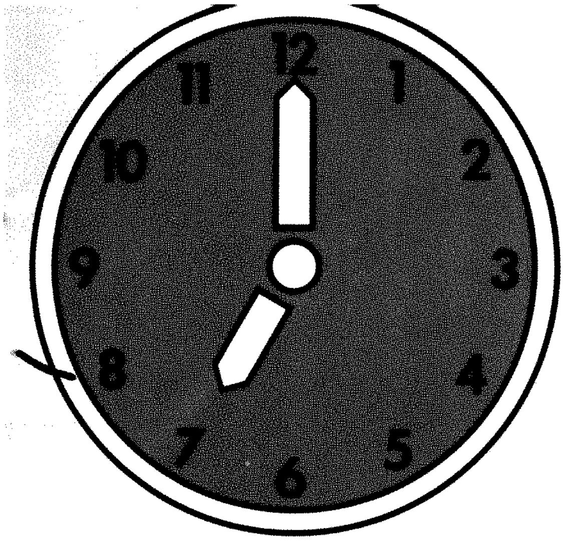
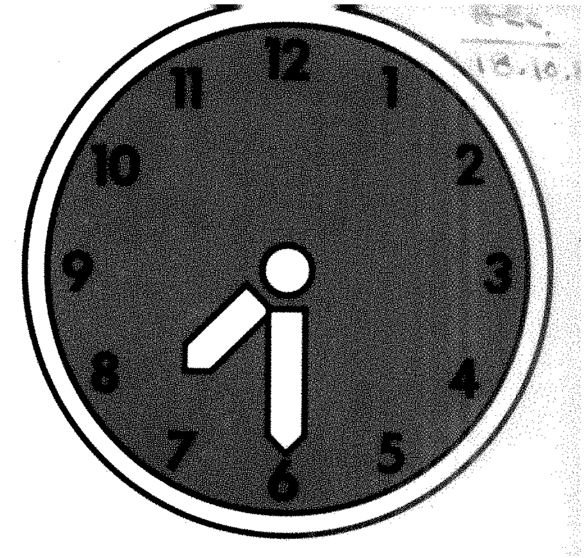


PUT IT ON



WASH IT OFF

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INDICATIONS
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DIRECTIONS FOR USE
Dithrocream may be applied at night before retiring and washed off in the morning Alternatively, it may be applied at any convenient time and then removed by washing off, usually no more than one hour after application. Continue treatment until the skin is smooth and clear.

CONTRA-INDICATIONS
Acute psoriasis, dithranol sensitivity

PRECAUTIONS
Keep away from the eyes. Apply to psoriatic areas only. Wash the hands after use

PACKAGE QUANTITY
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PRODUCT LICENCE NOS
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Dithrocream Forte (0.5%): 0173/0027.
Dithrocream HP (1.0%): 0173/0039

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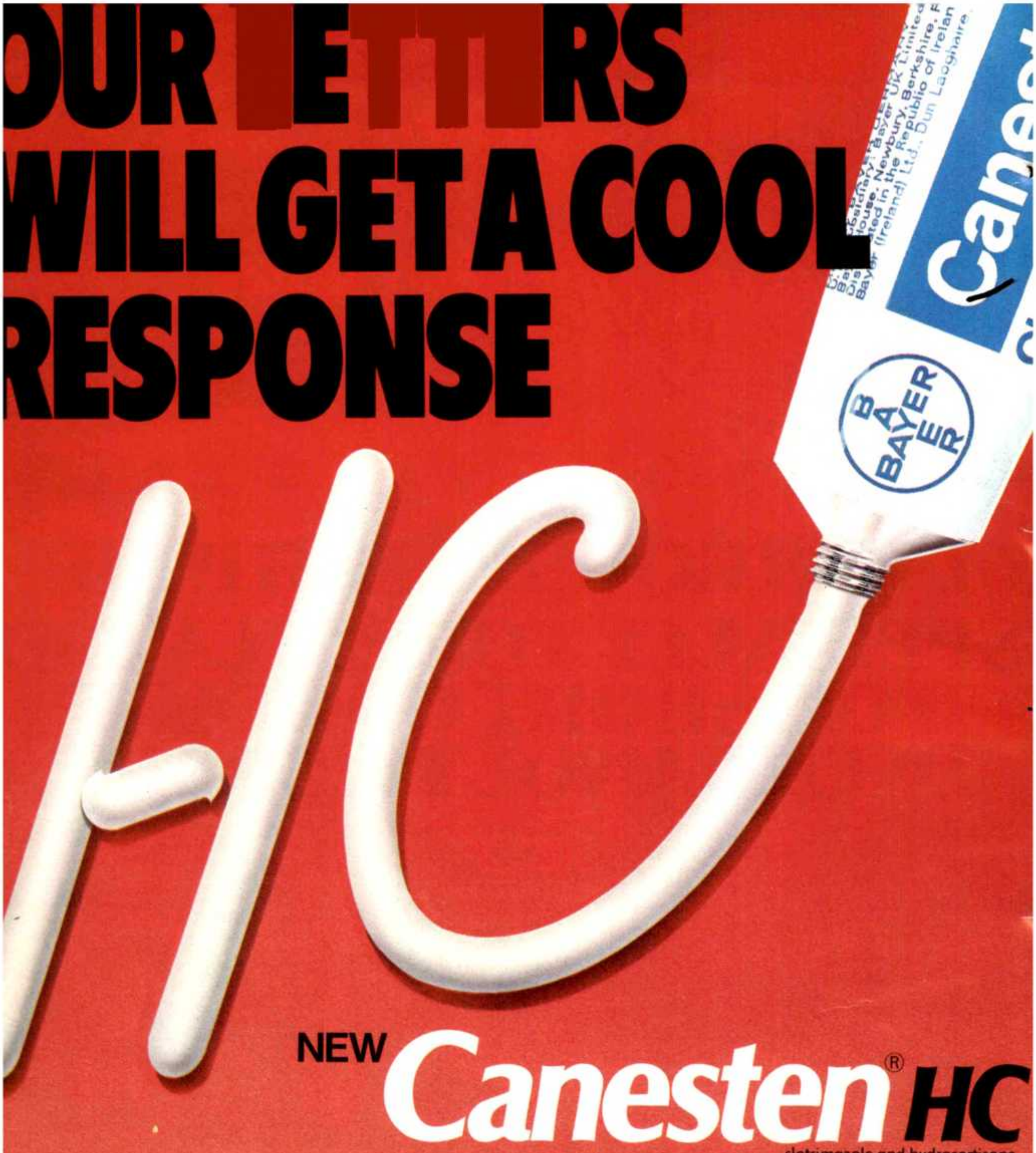

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clotrimazole and hydrocortisone

Canesten and 1% hydrocortisone for itchy, inflamed fungal infections

Prescribing Information – Presentation: A white cream containing 1% clotrimazole and 1% hydrocortisone. **Indications:** All fungal skin infections due to dermatophytes, yeasts, or moulds where there is co-existing inflammation requiring rapid relief. **Dosage and Administration:** Canesten HC should be applied twice daily to the affected areas, and rubbed in gently. **Contra-indications:** Hypersensitivity to any of the ingredients. **Side Effects:** Rarely, patients may experience mild local irritation or burning after application. **Precautions:** Care, as with any topical corticosteroid, is advised with infants and children where the preparation is to be applied to extensive surface areas or under occlusive dressing. However, continuous prolonged treatment should not be necessary. Topical steroids should not be used extensively in pregnancy. **Basic NHS Cost:** Cream 30g £3.18. **Product Licence Number:** 0010/0120

Further information is available from:
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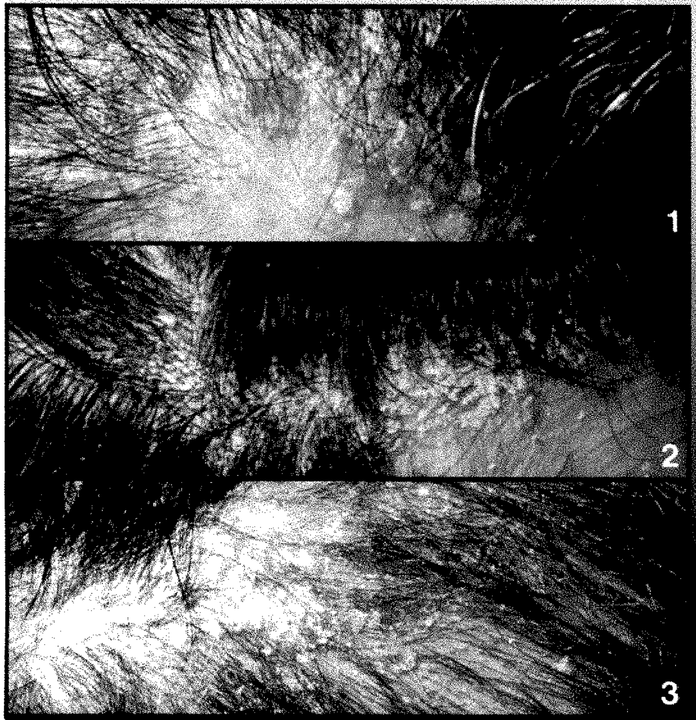
Common Disorders of the Scalp.

They all have one thing in common [illegible]



1. Psoriasis of the Scalp 2. Seborrhoeic Dermatitis of the Scalp 3. Pityriasis Capitis (Dandruff)

Polytar Liquid

Tar B.P., Cade Oil B.P.C., Coal Tar B.P.C., Arachis Oil extract of crude coal tar, Oleyl alcohol.

Acknowledged as ideal for long-term control.

ALSO Polytar Plus with protein

Prescribing Information.

Presentation Polytar Liquid is a concentrated, antiseptic tar-medicated scalp cleanser containing the following active ingredients:

Tar B.P.	0.3%
Cade Oil B.P.C.	0.3%
Coat Tar B.P.C.	0.1%
Arachis Oil extract of crude coal tar	0.3%
Oleyl alcohol	1.00%

Polytar Plus contains 3.0% hydrolysed animal protein, in addition to the above.

Uses Polytar Liquid and Polytar Plus are indicated in the treatment of scalp disorders such as psoriasis, seborrhoea, dandruff, eczema and pruritus. They are also of value in the removal of pastes and pomades used in the treatment of psoriasis.

Dosage and Administration The hair should be wetted and sufficient applied to produce an abundant lather. The scalp and adjacent areas should be vigorously massaged with the fingertips. The hair should then be thoroughly rinsed and the procedure repeated.
Polytar Liquid or Polytar Plus should be used once or twice weekly.

Contra-indications, warnings, etc. Nil

Pharmaceutical Precautions Store in a cool place

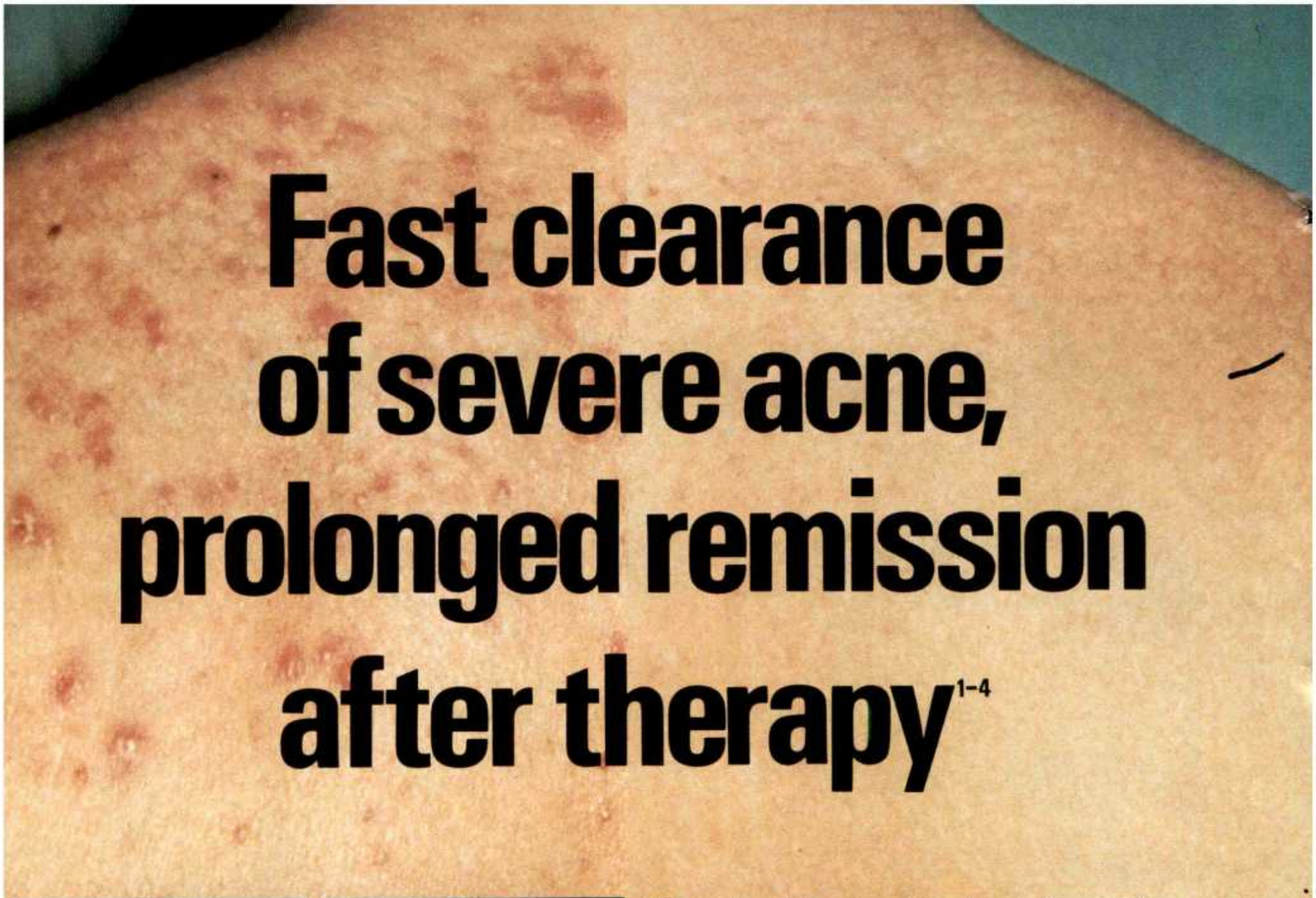
Packs and Prices

	Pack	Basic NHS price
Polytar Liquid	65ml	91p
	150ml	£1.34
	350ml	£2.37
	1 litre	£6.18
Polytar Plus	150ml	£1.43
	350ml	£2.80

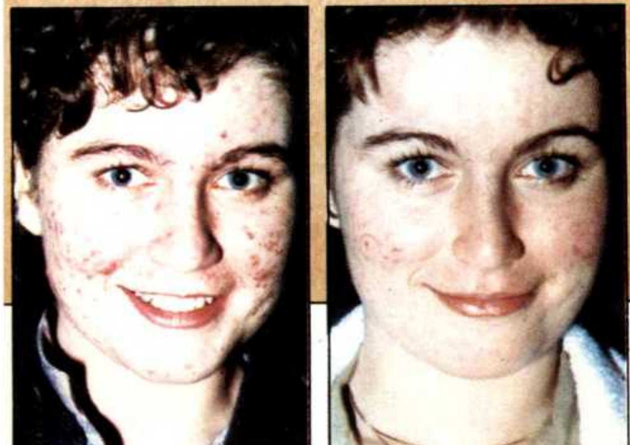
Product Licence Number
Polytar Liquid PL0174/5016 Polytar Plus PL0174/0037

Full information and Data sheet on request.

Stiefel Laboratories (UK) Limited
Wellcroft Road, Slough SL1 4AQ



Fast clearance of severe acne, prolonged remission after therapy[1-4]



"The aim of therapy is not only to produce clearing of active disease, but also to maintain this clearing indefinitely."[5]

In severe acne, Roaccutane rapidly produces an excellent response in 90% of patients and prolonged remissions after therapy once clearance is achieved.[1-4]

(Main picture) Male patient with truncal acne unresponsive to oral antibiotics, before and after a course of Roaccutane.

Female patient unresponsive to oral antibiotics, before and after a course of Roaccutane.

ROACCUTANE

isotretinoin

a turning point in acne therapy, for both male and female patients

BRIEF PRESCRIBING INFORMATION

Indications Cystic and conglobate acne, severe acne unresponsive to systemic antibiotics.

Dosage 0.5mg/kg daily for 12-16 weeks. Increase up to 1mg/kg daily if response is poor at 4 weeks. Reduce dosage if tolerance is poor. As a rule, other anti-acne therapy, including antibiotics, is not indicated. Repeat course of treatment should not normally be given.

Contra-indications Pregnancy (Roaccutane is teratogenic—see 'Precautions') and breast feeding. Hepatic or renal impairment.

Precautions The following precautions must be observed in women of childbearing potential.

1. Patients should be given detailed explanation of the risks.
2. Pregnancy should be excluded before therapy is instituted.
3. Effective contraception must be ensured for one month before treatment, during treatment, and for one month after treatment ends.

Use of patient information leaflets is strongly recommended. Monitor liver function and serum lipids (fasting value) regularly.

Side-effects Mainly mucosal dryness and fissures; facial dermatitis; desquamation; epidermal fragility; conjunctivitis; arthralgia/myalgia; increased hair loss (uncommon and reversible); possible elevation of liver enzymes and serum triglycerides. Benign intracranial hypertension has been reported, particularly in association with concomitant antibiotics.

Availability Hospitals and hospital clinics.

Presentations and Basic NHS Cost

Roaccutane capsules 5mg × 100 £33.56.

Roaccutane capsules 20mg × 100 £96.73.

Product Licence Numbers 0031/0158 (capsules 5mg), 0031/0160 (capsules 20mg).

Licence Holder

Roche Products Limited
PO Box 8, Welwyn Garden City
Hertfordshire AL7 3AY

References

1. In: "Retinoids: Advances in Basic Research and Therapy", Eds. Orfanos, C.E. *et al.*, Springer-Verlag, Berlin, 1981, p.219.
2. Brit. J. Derm., 1983, *108*, 333.
3. J. Amer. Acad. Derm., 1982, *6*, 735.
4. J. Amer. Acad. Derm., 1982, *6*, 766.
5. J. Amer. Acad. Derm., 1983, *9*, 629.

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ROCHE Retinoids **From Roche—world leader in retinoid research**

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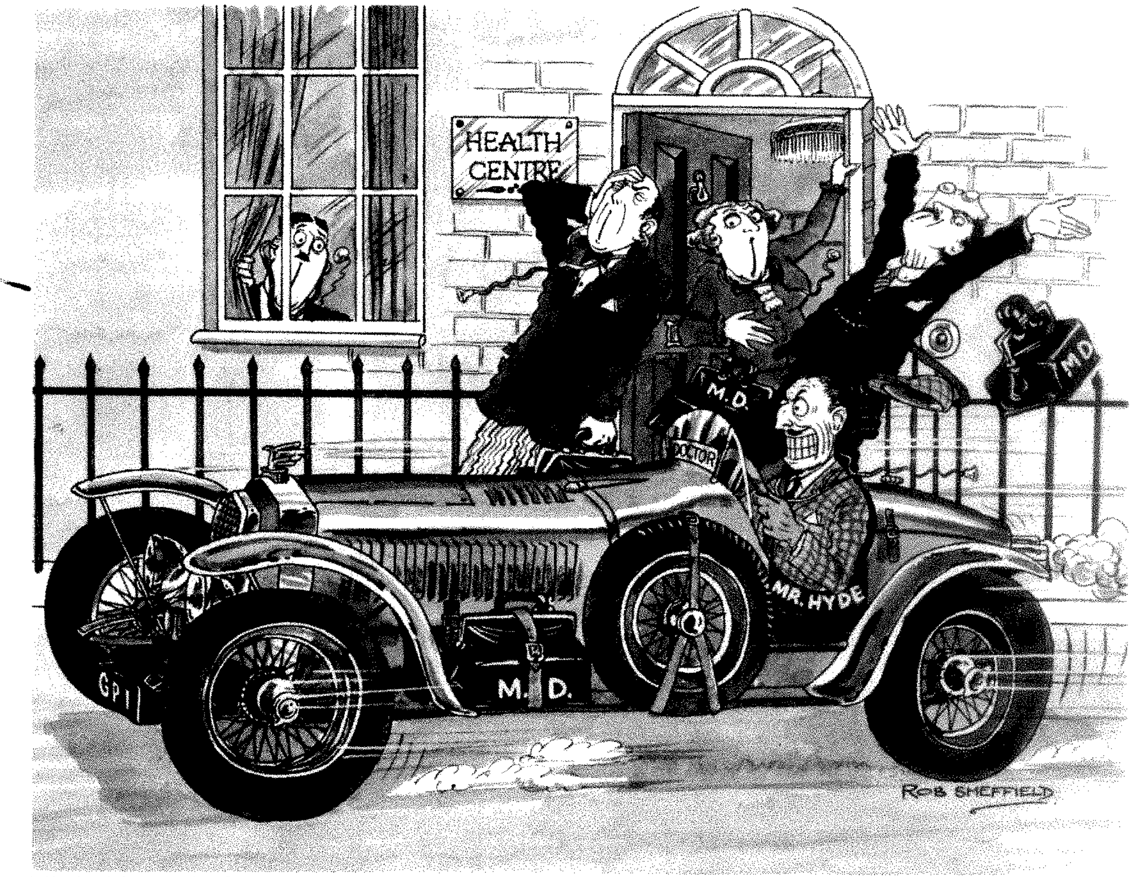

With acknowledgements to H.M. Bateman.

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Betnovate RD is available as cream or ointment in familiar and convenient 100 gram tubes.

Betnovate RD

(betamethasone valerate)

Simply Betnovate, Ready Diluted

Prescribing information

Presentation

Betnovate RD Cream and Ointment contain betamethasone valerate ready-prepared in a one-in-four dilution, giving a strength of 0·025%.

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Stratum Corneum

Editors: **R. Marks, G. Plewig**

1983. 106 figures, 41 tables. XI, 265 pages.
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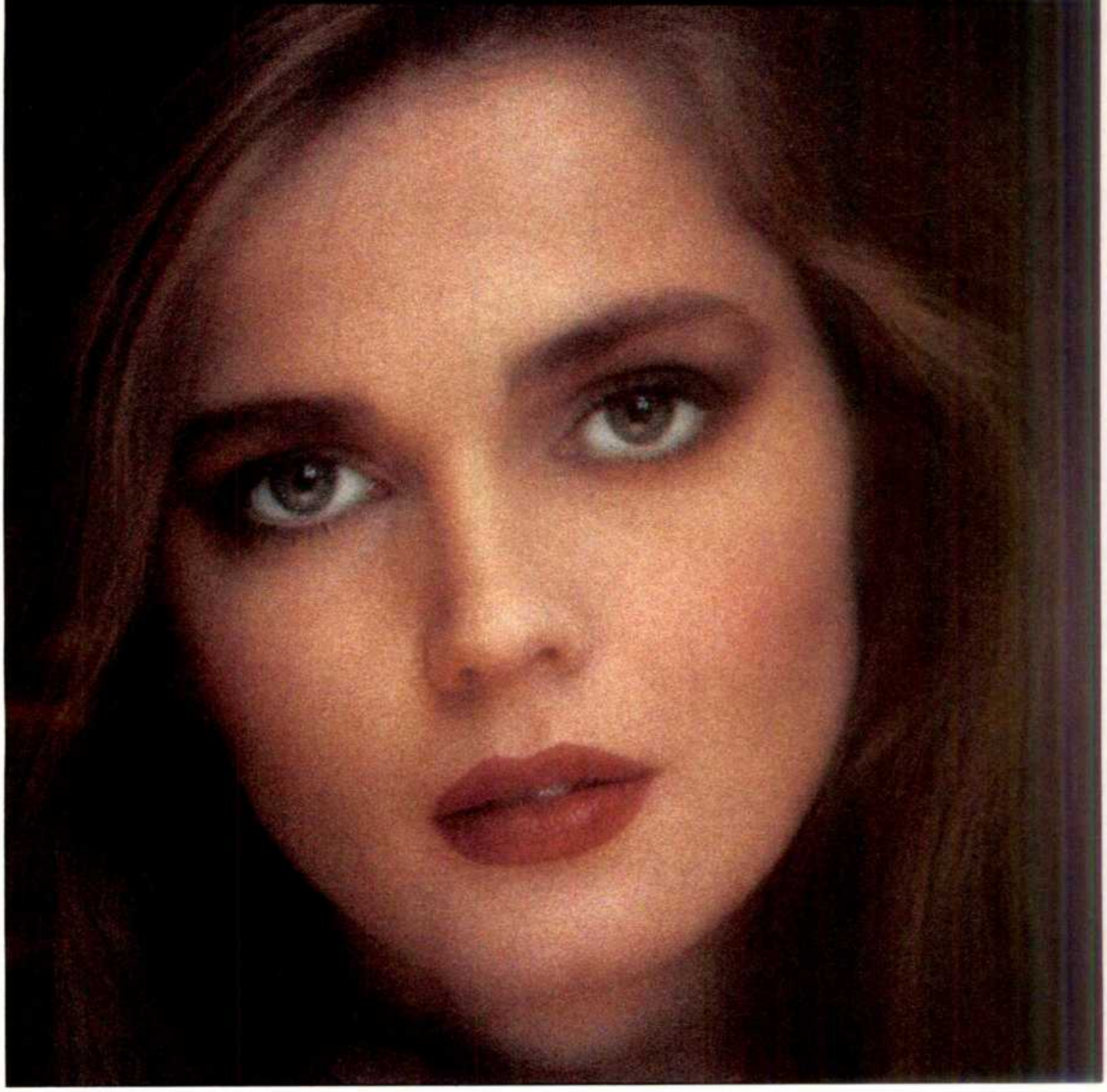

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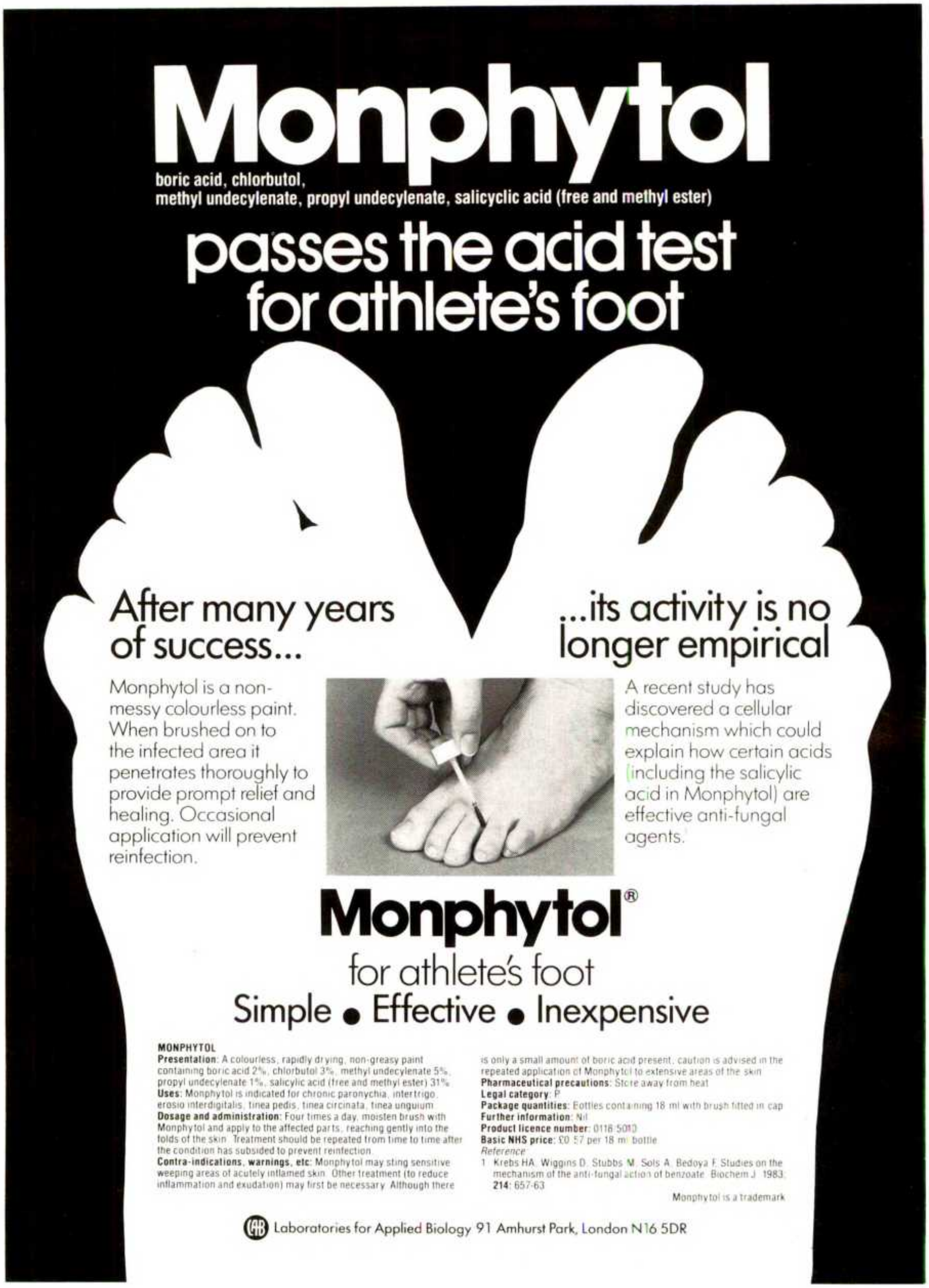

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1 Krebs HA, Wiggins D, Stubbs M, Sols A, Bedoya F. Studies on the mechanism of the anti-fungal action of benzoate. Biochem J. 1983; **214**: 657-63

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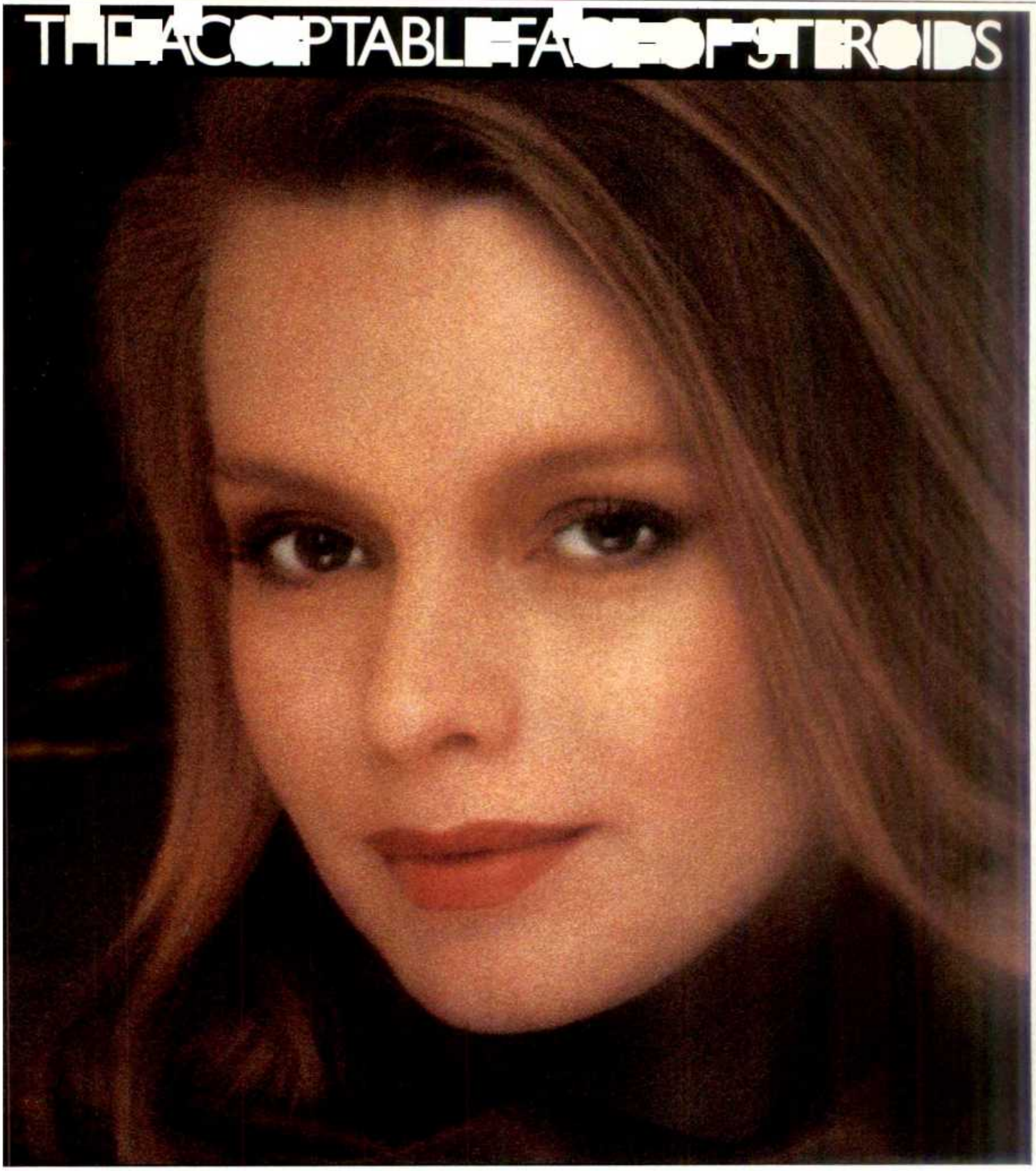

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British Journal of Dermatology (1984) 111, 253–264.

Clinical and Laboratory Investigations

Effect of enzyme digestion on the permeability barrier in keratinizing and non-keratinizing epithelia

C.A.SQUIER

Dows Institute for Dental Research, College of Dentistry, The University of Iowa, Iowa City, IA 52242, U.S.A.

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SUMMARY

In an attempt to characterize the permeability barrier of the oral mucosa and skin, small pieces of keratinized and non-keratinized oral epithelia and epidermis were digested with specific enzymes. These enzymes were selected for their effect on carbohydrate–protein, or carbohydrate–lipid compounds and phospholipids. The effect of the enzyme treatment was monitored by exposing the digested tissue to horseradish peroxidase. Electron microscopic examination of tissue treated with phospholipases revealed considerable damage to membrane structures but not to the integrity of the permeability barrier. Hyaluronidase and neuraminidase caused less structural damage but did not impair barrier function; this was only seen after treatment with chondroitinase ABC. This enzyme may degrade certain of the polar molecules thought to be necessary to stabilize the neutral lipid bilayers of the intercellular barrier and thus disrupt its barrier properties.

During the past decade there has been a considerable increase in our knowledge of the macromolecular basis of the epidermal permeability barrier in mammalian skin. Measurements of percutaneous perfusion combined with stripping and separation experiments have long suggested that the barrier was located in the stratum corneum (Monash & Blank, 1958; Eriksson & Lamke, 1971). Schreiner & Wolff (1969) using ultrastructural tracers demonstrated an intercellular barrier in the stratum corneum and suggested that a specific organelle, the membrane coating granule, might be associated with formation of this barrier. Subsequent tracer and freeze-fracture studies confirmed these findings (Elias & Friend, 1975; Elias, McNutt & Friend, 1977). More recently it has been claimed that the intercellular region represents the principal barrier to transepidermal penetration (Elias, 1981) and is composed of a variety of free fatty acids, polar and neutral lipids and possibly glycolipids (Gray & Yardley, 1975; Elias *et al.*, 1979; Wertz & Downing, 1982) rather than phospholipids, as originally proposed (Hashimoto, 1971; Hayward, 1978).

In contrast to the well-defined barrier function of epidermis, the stratified squamous epithelium lining the oral cavity has often been considered to be a permeable tissue (Malkinson, 1964; Winkelmann, 1969), like other regions of the alimentary tract. Some areas of the oral cavity are non-keratinized resembling oesophagus or uterine cervix, but there are also keratinized regions with a structure similar to that of epidermis (Squier, Johnson & Hopps, 1976). Studies using water-soluble ultrastructural tracers such as horseradish peroxidase and lanthanum have revealed the presence of an intercellular permeability barrier in the superficial layers of both keratinized and non-keratinized oral epithelia (Squier, 1973; Squier & Rooney, 1976). In the keratinized regions of oral mucosa this barrier appears to be derived from the lamellate contents of membrane coating granules in a similar manner to the process described in the epidermis. Non-keratinized oral epithelium possesses an homologous population of granules which may also be associated with formation of a barrier in the superficial layer of the tissue (Squier, 1977).

There has been no chemical characterization of the barrier in oral epithelium, but given the similarities between epidermis and keratinized and non-keratinized oral epithelium when exposed to ultrastructural tracers, it is appropriate to ask whether the permeability barrier might be similar at a macromolecular level. This question has been approached in the present study by treating epidermis and oral epithelium with specific enzymes and then using horseradish peroxidase as an intercellular probe to reveal any changes in permeability. Differential enzyme digestion has been previously applied to ultrathin sections of fixed and resin-embedded epidermal specimens (Wolff & Schreiner, 1971), but this procedure is not helpful for examining changes in lipid-containing substances which are susceptible to the action of many of the solvents used for electron microscopic preparation. However, Kanwar, Linker & Farquahar (1980) were able to identify the compounds responsible for filtration in the glomerular basement membrane by perfusing fresh kidney with enzyme solutions followed by the use of electron-dense tracers.

METHODS

Small biopsies of skin and oral mucosa were removed under general anaesthesia from the sites and animal species indicated in Table 1. Each biopsy was immediately divided into 1 mm cubes which were then placed in the buffered enzyme solutions listed in Table 2. These enzymes were selected by reason of their action on two types of compound: (1) those in which carbohydrates

TABLE 1. Sites and source of tissue (values indicate number of animals used)

	Tissue region			
	Keratinized		Non-keratinized	
Species	Skin	Palate	Cheek	Lip
Rabbit	—	3	5	—
Rat	2	2	—	—
Pig	2	2	—	—
Monkey	2	4	2	1

TABLE 2. Source and preparation of enzymes used

Enzyme	Source*	Preparation
Neuraminidase	*Cl. perfringens*	0·1 mg/ml in 0·1 M phosphate buffer at pH 6·0
Hyaluronidase	Ovine testicular	500 i.u./ml in 0·1 M phosphate buffer at pH 6·0 containing 0·15 M NaCl
Chondroitinase ABC	*P. vulgaris*	5 units/ml in 0·1 M sodium acetate buffer at pH 6·5 5 units/ml in 0·1 M Tris–HCl buffer at pH 7·5 5 units/ml in 0·1 M Tris–HCl buffer at pH 8·2
Phospholipase A	*Crotalus terrificus*	1 mg/ml in 0·1 M Tris maleate buffer at pH 6·5 containing 1 mM $CaCl_2$
Phospholipase C	*Cl. Welchii*	0·1 mg/ml in 0·1 M Tris–maleate buffer at pH 7·3

* All from Sigma Chemicals, St Louis, Mo., U.S.A.

are attached to protein or lipid moieties (neuraminidase, hyaluronidase and chondroitinase ABC) and (2) phospholipids (phospholipase A, phospholipase C).

After incubation in the presence of the enzyme for 1 hour at 37°C with gentle agitation, the specimens were briefly rinsed in the respective buffers and then incubated with horseradish peroxidase (HRPO) in 0·9% saline (Sigma Type VI; 30 mg/ml) for 1 hour at 37°C with agitation. All specimens were fixed in 2% formaldehyde/2·5% glutaraldehyde (Karnovsky, 1965) in 0·08 M cacodylate buffer at pH 7·3 for 20 minutes and then rinsed in three changes of the same buffer. For light microscopy, the tissue blocks were mounted on cork disks and snap-frozen in isopentane cooled in liquid nitrogen. Sections were cut at 6 μm in a cryostat at −15°C and collected on coverslips. Tissue destined for electron microscopic examination was embedded in 6% agar and chopped at 30–40 μm on a Sorvall tissue chopper (Dupont Instruments, Norwalk, Conn.). Sections were always taken 20–30 μm beneath the surface of the tissue block to avoid mechanical damage arising from the original division of the tissue biopsy (Squier & Hopps, 1976). The localization of the HRPO was revealed by incubating all specimens in a Tris–maleate buffered solution containing diamino-benzidine and hydrogen peroxide (Graham & Karnovsky, 1966). After rinsing in distilled water, sections for light microscopy were mounted in an aqueous medium.

For electron microscopy, the specimens were rinsed in distilled water, fixed in 2% aqueous osmium tetroxide for 1 hour, dehydrated in ethanol and embedded in Spur's epoxy resin. Ultrathin sections were cut and examined in a Siemens Elmiskop electron microscope with, and without, heavy metal counter-staining.

Controls

Some cryostat sections were stained with haematoxylin and eosin to permit evaluation of the tissue structure.

Controls for enzyme digestion:

(1) Specimens were incubated in buffer without enzyme before transferring to HRPO and processing as described.

(2) After enzyme treatment some tissue blocks were processed directly for electron microscopic examination to assess the effect on the fine structure and thus to confirm penetration by the enzyme.

Controls for HRPO activity:

(1) Some specimens were incubated directly in HRPO without enzyme pretreatment and then processed as described.

(2) Endogenous enzyme activity; we have previously shown that no endogenous peroxidase activity can be detected within the epithelium (Squier & Hopps, 1976).

RESULTS

Light microscopic observations

The tissues examined represented keratinized oral epithelium (Fig. 1a), epidermis (Fig. 2a) and non-keratinized oral epithelium (Fig. 3a); these differed in the overall thickness of the epithelium and of the keratinized layer, where present. Sections from control blocks which had

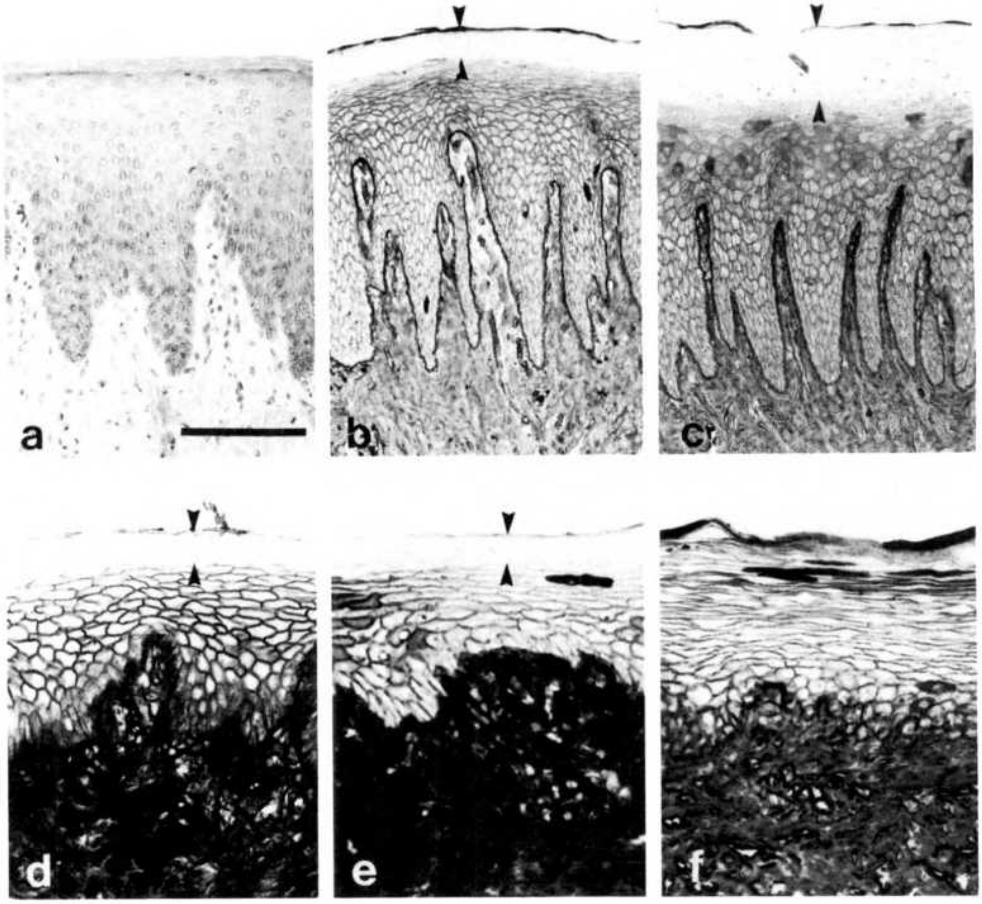

FIGURE 1. (a) Rabbit palate stained with haematoxylin and eosin; (b) incubated with HRPO only; (c) treated with hyaluronidase and incubated with HRPO. The deeper boundary of the permeability barrier is very indistinct. (d) Pig palate incubated with HRPO only; (e) treated with phospholipase and incubated with HRPO (note leakage of tracer into many of the nucleated cells but not across the superficial barrier); (f) treated with chrondroitinase ABC and incubated with HRPO. Tracer has penetrated through the entire barrier as compared with (d). All micrographs at same magnification; bar represents 100 μm. Arrowheads indicate approximate extent of the barrier layer.

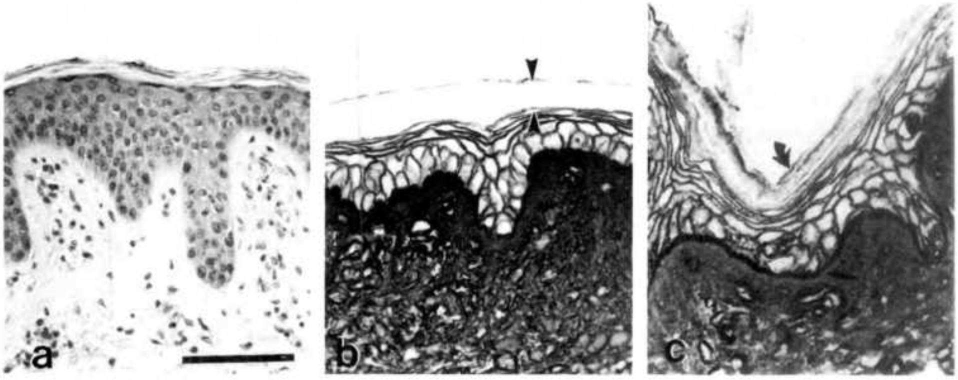

FIGURE 2. Pig skin (a) stained with haematoxylin and eosin; (b) incubated with HRPO only; (c) treated with chondroitinase ABC and incubated with HRPO. Note the penetration of tracer through the barrier layer (arrow) as compared with (b). All micrographs at same magnification; bar represents 50 μm. Arrowheads indicate approximate extent of the barrier layer.

been incubated directly in HRPO revealed the normal extent of the permeability barrier in each of the tissues (Figs 1b, 1d, 2b, 3b).

In examining the enzyme-treated tissues, tracer was often seen within cells located in the deeper, nucleated cell layers. Such intracellular localization of HRPO was sometimes also seen in buffer controls and it is assumed that this represents non-specific damage arising from incubation at non-physiological pH or in non-physiological solutions.

All keratinized epithelia responded similarly to any one enzyme. Neuraminidase and hyaluronidase had similar but minimal effects on the integrity of the barrier, the most obvious feature being the indistinct deep margin instead of the sharp boundary seen in control sections (Figs 1b, 1c). Neither of the phospholipases permitted the tracer to penetrate the superficial

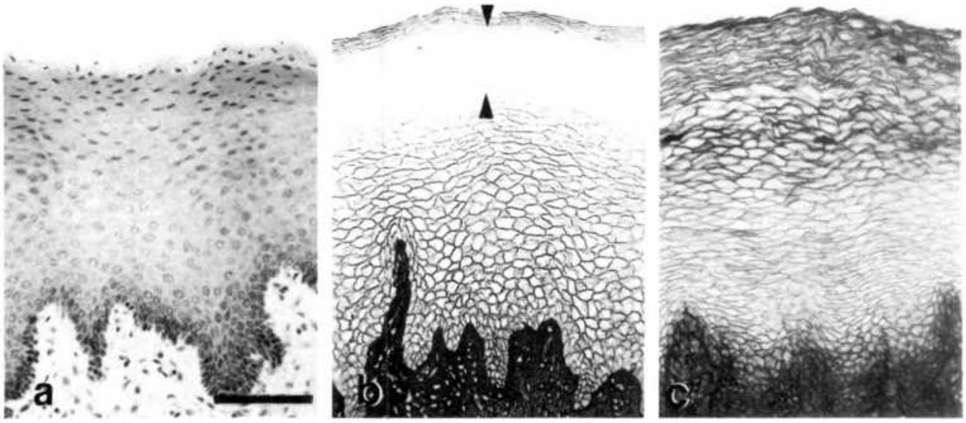

FIGURE 3. Rabbit buccal mucosa (a) stained with haematoxylin and eosin; (b) incubated with HRPO only; (c) treated with chondroitinase ABC and incubated with HRPO. All micrographs at same magnification; bar represents 100 μm. Arrowheads indicate approximate extent of the barrier layer.

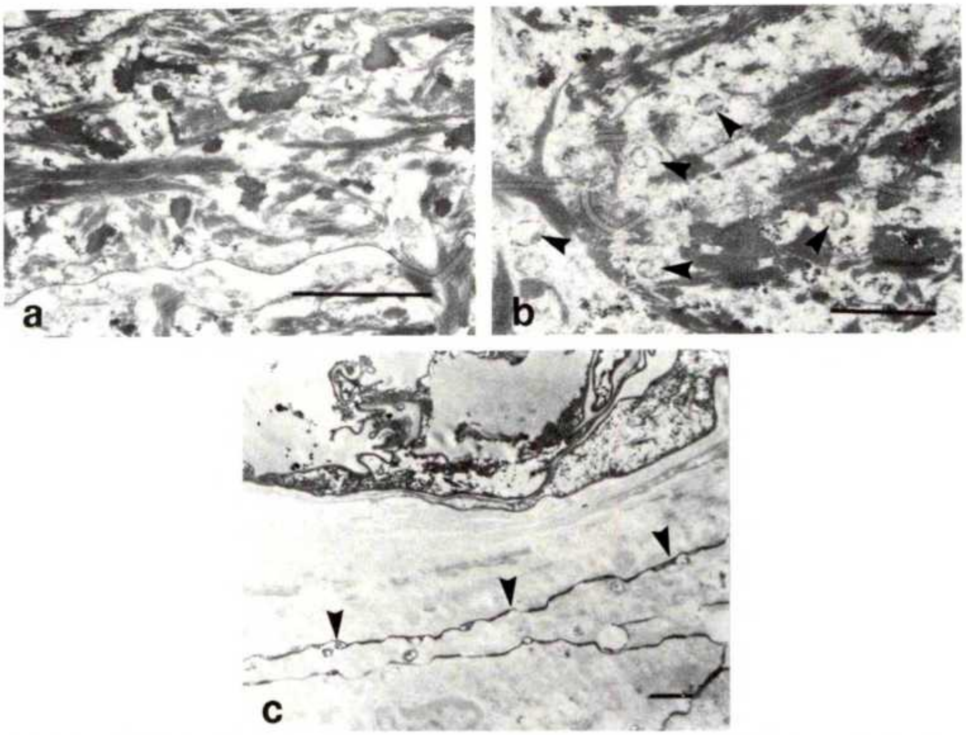

FIGURE 4. Electron micrographs of granular layer/stratum corneum of rat palate epithelium after incubation with phospholipase A. (a) Plasma membranes, but not the intracellular thickening, have been lost; amorphous material is present in the intercellular spaces. (b) Membrane coating granules lacking internal lamellae are evident (arrows) adjacent to the cell margins. (c) Rat epidermis after treatment with phospholipase C and incubation in HRPO. Despite the enzyme treatment, tracer has not penetrated beyond the upper granular layer/stratum corneum (arrows). The superficial, desquamating, squames appear to have hydrated and ruptured. Bars represent 1 μm.

barrier region (Figs 1e, 4c) although there was often leakage of nucleated cells (Fig. 1e). The only enzyme with an obvious effect on the superficial barrier was chondroitinase ABC. Of the three pH optima used with chondroitinase ABC, that of 7·5 brought about the greatest effect. After treatment of the tissue with this enzyme, tracer penetrated intercellularly through the full thickness of the keratinized layer (Figs 1f, 2c), although there were some differences between different regions and species. For example, the barrier region in parakeratinized epithelium in the palate was usually more completely permeated than that of the orthokeratinized oral regions and epidermis, and porcine tissues were more resistant than those of the other animals examined. Tracer was also sometimes seen within the keratinized squames after chondroitinase treatment (Fig. 1f).

Non-keratinized oral epithelia and keratinized tissues showed a similar spectrum of changes when exposed to the enzymes, although there was a greater tendency for tracer to leak into cells of non-keratinized tissue, all of which are nucleated; this was often seen in the most superficial cell layers. Neither neuraminidase, hyaluronidase nor the phospholipases damaged the entire barrier region of non-keratinized epithelium, whereas treatment with chondroitinase ABC always permitted HRPO to penetrate through the full thickness of the epithelium (Fig. 3c).

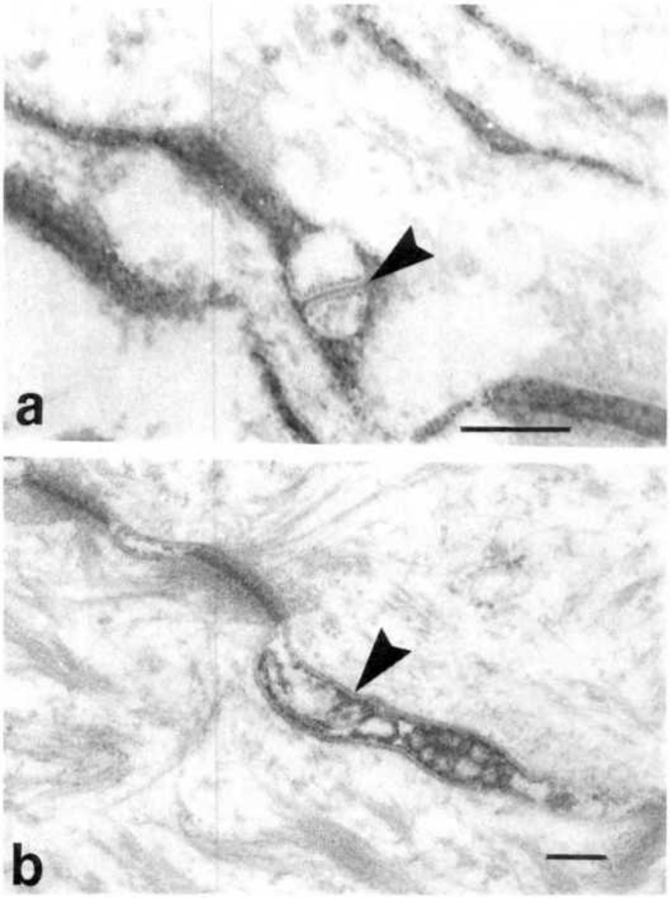

FIGURE 5. Granular layer of rabbit palate treated with chondroitinase ABC and incubated in HRPO. A distinct intercellular lamella (arrow) is present in (a) and several lamellar structures (arrows) are seen in (b). Electron dense tracer has penetrated through the intercellular spaces. Bars represent 0·1 μm.

Electron microscopic observations

In order to determine whether the enzymes had adequately penetrated the treated tissues, specimens were examined in the electron microscope after incubation with each of the enzymes alone. All such specimens showed some evidence of damage at the cellular level; this was minimal after treatment with hyaluronidase, neuraminidase and chondroitinase ABC whereas the phospholipases caused total destruction of membranes, including those of organelles and of the cell (Figs 4a, 4b). Membrane coating granules, identified by their location at the superficial margin of cells in the granular layer, showed loss of the characteristic parallel lamellae (Fig. 4b) and no intercellular lamellae were evident at the junction of the granular layer and stratum corneum (Fig. 5a). Despite this damage, tracer did not penetrate the superficial permeability barrier when phospholipase-treated tissue was incubated in HRPO and examined with the electron microscope (Fig. 4c).

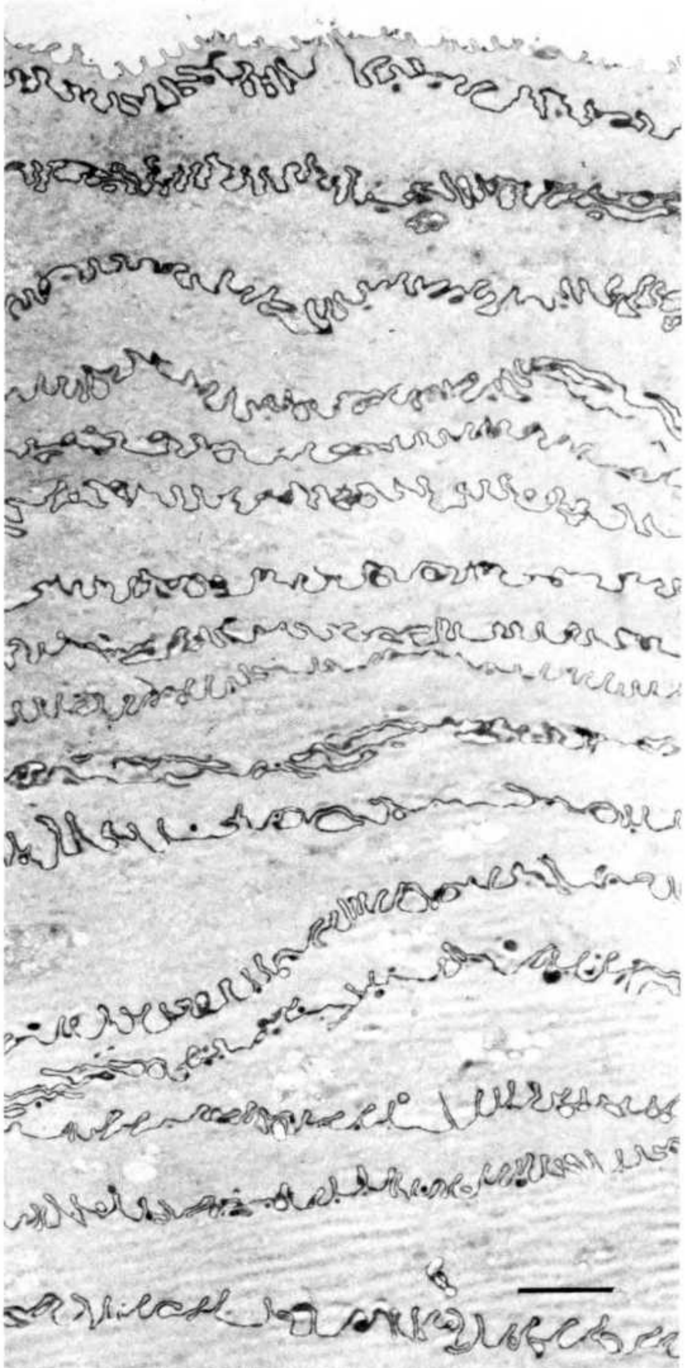

FIGURE 6. Superficial cell layers of rabbit buccal epithelium treated with chondroitinase ABC and incubated in HRPO. Tracer has penetrated between every cell layer (compare with Fig. 3c). Bar represents 1 μm.

In tissue treated with chondroitinase ABC this state of affairs was reversed; many membranous organelles were intact and some lamellate structures could be identified in the intercellular spaces of the superficial granular layer (Fig. 5). Nevertheless, HRPO was able to penetrate beyond such lamellae into the intercellular regions of the keratinized layer and often extended as far as the surface. Non-keratinized epithelia after chondroitinase treatment permitted penetration of HRPO through the full thickness of the superficial region (Fig. 6).

DISCUSSION

In interpreting the results of this study it must be remembered that digestion with a limited number of specific enzymes is a relatively crude technique by which to characterize chemically the epithelial permeability barrier. Nevertheless, when combined with electron microscopy, the procedure permits the effects of the enzymes to be spatially localized with a precision not often provided by biochemical methods. As the buffer control preparations indicated, the incubation of unfixed tissue does in itself cause some tissue damage, manifested principally as leakage of tracer into the nucleated epithelial cells. However, this never compromised the integrity of the superficial epithelial barrier, which is in agreement with the results of *in vitro* permeability studies where consistent measurements can be obtained after holding tissue in buffered solutions for long periods (Galey, Lonsdale & Nacht, 1976).

Another question is whether the enzymes that were without effect on the permeability barrier were able to gain access to it adequately. Two pieces of evidence would suggest that the enzymes were not restricted in their penetration. First, there was microscopic evidence of damage by those enzymes which did not affect the permeability barriers (Fig. 4). Secondly, the enzyme with the greatest effect, chondroitinase ABC, also has the highest molecular weight (150,000 daltons; Yamagata *et al.*, 1968), whereas phospholipase and hyaluronidase, which both had minimal effect on the barrier, have molecular weights of 15,000 daltons or less (Boyer, 1971; Boyer, Hardy & Myrbask, 1966). Although molecular weight is only one factor influencing penetration of a substance, it would seem unlikely that only the largest molecule gained access.

One of the major differences between skin and oral mucosa is the moist surface of the latter, arising from salivary secretions. Although mucus has been claimed to have a barrier effect (MacPhee & Cowley, 1969), Adams (1974) found that adding saliva to oral mucosa maintained in perfusion chambers caused no significant change in permeability. Nevertheless, although it may not affect the functioning of the barrier, the loss of a surface mucous layer as a result of incubation *in vitro* may have facilitated the penetration of tracer from the exterior into the most superficial cells, which was most frequently seen in non-keratinized epithelium. Apart from this feature the various enzymes had a similar effect on epidermis and keratinized oral epithelium.

Neuraminidase and hyaluronidase were without marked effect on the intercellular epithelial barrier of skin and oral mucosa. These enzymes might be expected to degrade proteoglycans, which have been shown to be the major component of the intercellular substance in oral epithelium (Wiebkin, Bartold & Thonard, 1979; Bartold, Wiebkin & Thonard, 1981) yet do not seem to be a functional constituent of the superficial barrier layer.

It is not surprising that the epidermal permeability barrier was unaffected by the action of phospholipases, for there is general agreement that phospholipids are absent from the epidermal stratum corneum. The similar results obtained after treating keratinized oral epithelium with phospholipases suggest that this tissue may also lack phospholipids in its surface layer, although no specific lipid analyses have yet been reported.

The mechanism by which chondroitin ABC increased epithelial permeability to HRPO is open to speculation. The material making up the permeability barrier in epidermis is thought to consist of intercellular arrays of neutral lipids, probably derived from the membrane coating granules (Elias *et al.*, 1979). These are probably arranged as bilayers and may be stabilized by the presence of polar compounds such as gangliosides, glucosyl ceramide, cholesterol sulphate, glycolipids and glycoproteins. However, there is considerable debate as to the relative contribution and persistence of such compounds in the barrier (Elias *et al.*, 1979; Wertz & Downing, 1982, 1983). Chondroitinase ABC cleaves acetylgalactosaminyl bonds of substrates such as chondroitin sulphate and dermatan sulphate to yield sulphated dissaccharides (Yamagata *et al.*, 1968; Suzuki *et al.*, 1968). It is thus possible that this enzyme has an effect on the sulphated glycoproteins (and possibly glycolipids; D.Downing, personal communication) which may contribute the polar groups that stabilize the intercellular barrier material. Removal of these groups would disrupt the bilayer arrangement and so weaken its barrier properties. Testicular hyaluronidase is also capable of degrading chondroitin sulphate and dermatan sulphate, so it is unclear why this enzyme has so little effect. One reason may be that the larger fragments (octasaccharides; Silbert, 1966) remaining after hyaluronidase digestion still contribute to the integrity of the barrier.

Of interest are the electron micrographs of keratinized tissue (Fig. 5) incubated with chondroitinase ABC, which show intact lamellar structures between the cells despite the penetration of tracer through the entire superficial barrier region. These structures may represent expelled lamellae that have not yet reorganized to form an effective stable barrier structure and so would not be expected to restrict permeability. This would be in accord with suggestions of an alteration or maturation of the granule contents after their expulsion from the cell and prior to barrier formation (Elias *et al.*, 1977). On the other hand, even when lamellae are not evident microscopically in the membrane coating granules, as in the phospholipase-treated tissue, a functional barrier can exist. Presumably the stabilized bilayers of the barrier that exists between the keratinized squames do not always bind sufficient osmium to be visible in the electron microscope in routine preparations. However, they can be demonstrated by special procedures (Elias *et al.*, 1979).

Finally, it is worth noting that the permeability barrier demonstrated in non-keratinized oral epithelia forms without the involvement of lamellate structures. The membrane coating granules in this tissue possess a core of amorphous material (Squier, 1977; Hayward, 1979) that has been shown by cytochemical staining to consist of glycolipid and/or glycoprotein (Hayward, 1973; Hayward & Hackmann, 1973). If this material is involved in barrier formation, then chondroitinase would have a direct effect on permeability of the epithelium.

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A simplified method for measurement of desquamation using dansyl chloride fluorescence

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SUMMARY

A fluorescence comparator has been used to assess quantitatively the skin fluorescence due to applied dansyl chloride. Experiments were performed demonstrating that measurements with the device had a low intra- and inter-observer error, with coefficients of variation of between 4% and 6%. Another experiment in which fluorescence photographic photometry was simultaneously performed showed that the comparator provided estimates of stratum corneum renewal similar to the more complex photographic technique. Repeated mechanical trauma to the skin and skin stripping showed that the technique can detect alterations in the rate of cell renewal in the epidermis.

We still know little of the effects of drugs or disease on epidermal cell renewal and differentiation. A major reason is the inadequacy of available techniques for quantifying desquamation. A suitable technique would be sensitive, reproducible, convenient, safe and able to provide multiple observations on the same individual. These are difficult criteria to meet for any sort of physiological measurement and especially difficult when it comes to measurement of a process that is difficult to monitor.

The dansyl chloride fluorescence method (Jansen, Hojyo-Tomoko & Kligman, 1974) for determination of the stratum corneum renewal time meets some, but not all, of the criteria mentioned. This paper describes a simple comparator technique for measurement of dansyl chloride fluorescence which we believe will be useful for the measurement of stratum corneum renewal and epidermal cell renewal.

METHOD

The comparator

The instrument used (Fig. 1) was constructed in the workshops of the Department of Medical

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Based on a paper presented at the Symposium of the International Society for Bioengineering and the Skin in Besançon, France, September 1983.

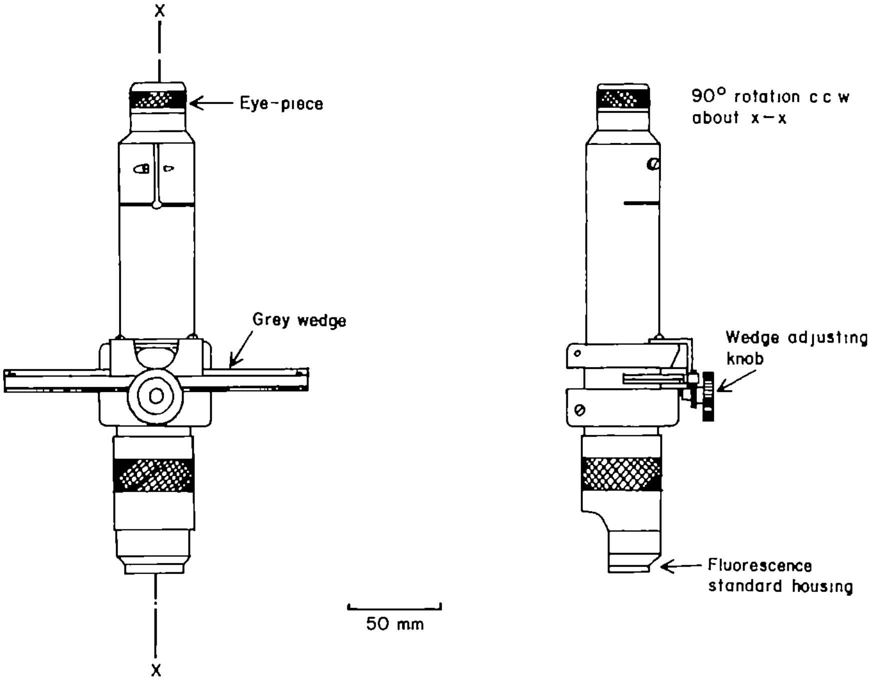

FIGURE 1. Diagrams to show construction of fluorescence comparator.

Physics in the University Hospital of Wales. This device possesses a rectangular plastic strip enclosing a portion of film which decreases from high to low density (a grey wedge). This occludes half of the lumen of the tube that supports it and can be made to travel along its length by a simple rack and pinion arrangement driven manually by a milled wheel. Its position can be recorded by a scale marked off in mm attached to its free edge. The 'grey wedge' moves over a fluorescent standard fixed to the bottom of the tube. This standard is obtained by taking a skin surface biopsy (Marks & Dawber, 1971) from an area of skin previously treated with 5% dansyl chloride (DC) in white soft paraffin (WSP). The skin surface biopsies used as standards were cut to size and slotted into the comparator instrument. Checks made by repeat fluorescence photographic photometry showed that fluorescence did not decline over a 2-month period.

In use the instrument is placed over the DC-treated test area of skin with the standard fluorescence adjacent to it, while both are illuminated by a hand-held UVA-emitting fluorescent lamp (Wood's light) placed between 4 and 8 inches away from the site being examined. Through a simple eye-piece lens the test area and the fluorescent standard can be viewed simultaneously as equal halves of a circle divided by a septum in the tube. The grey wedge is then adjusted so as to attenuate the brightness of the standard and appear to match in intensity. The reading on the wedge calibration is then noted.

Experiment to determine inter- and intra-observer error

Three normal healthy volunteer subjects had 5% DC in WSP applied under Finn chambers to two sites, one on either forearm (flexor aspect), for 24 hours. Six days after removal of the Finn chambers each site was examined by two observers (independently) five times with the comparator. Observations were repeated on days 7 and 11.

Experiment to compare the comparator method with a fluorescence photographic photometry technique

Ten normal healthy female subjects (age range 20–46 years, mean age 34·3 years) had 5% DC in WSP applied in Finn chambers to the middle of the flexor aspect of the forearm for 24 hours. At the end of the application period comparator readings were made from the test sites, using skin surface biopsies from half of the treated area as standard. Immediately after the comparator readings, fluorescence photographs of the sites were made using a standard ultraviolet fluorescence procedure (Finlay, Marshall & Marks, 1982). The negatives were developed in a standardized way and the density of the patch due to fluorescence was measured in a densitometer. From the density measurements the relative fluorescence measurements were made of the patch. Further measurements were taken at days 5, 7, 9, 12, 14 and 16 or until no further fluorescence was observed.

Experiment to assess effect of trauma and stripping on disappearance of fluorescence

In this experiment the effects on stratum corneum renewal of graded amounts of abrasive trauma applied to the forearm skin were investigated and compared with the effects of adhesive tape stripping. Four normal healthy volunteer subjects (mean age 28 years, age range 23–32 years), who had given their informed consent, had 5% DC in WSP applied to sites on the flexor aspects of their forearms. A specially constructed device (Marks, 1983) applied an abrasive stimulus to the forearm by mechanically driving a renewable plastic pad over the skin surface in a reciprocating motion. The trauma was applied twice daily for 4 days for periods of 20, 10 and 4 minutes in total to each site. A further site was partially stripped of stratum corneum by applying transparent adhesive tape (Sellotape) 15 times. The day before the trauma was started, each site to be investigated and a control site had 5% DC in WSP applied occlusively in Finn chambers. Readings were taken with the comparator at each site on days 0, 1, 2 and 3.

RESULTS

Inter- and intra-observer error

The results for both observers for each time point and for the six sites investigated are given in Table 1. Each of the values is the mean of five observations. The standard deviations of these

TABLE 1. Comparator readings for two independent observers on three subjects—two sites per subject. (Arbitrary units, means of five replicates ± s.d.)

	Subject 1		Subject 2		Subject 3	
	L	R	L	R	L	R
Observer A						
Day 6	130·2±6·1	110·4±5·2	117·6±3·6	102·6±4·6	103·2±4·8	94·2±5·1
Day 7	126·0±5·3	131·2±6·4	102·2±4·1	101·2±5·2	125·0±4·1	123·8±4·3
Day 11	99·8±6·1	101·0±5·4	95·6±5·8	95·8±5·5	75·6±4·1	—
Observer B						
Day 6	107·8±2·0	111·8±6·8	103·0±5·1	101·4±4·3	96·4±3·9	93·8±5·0
Day 7	106·0±9·0	116·2±7·8	102·2±4·1	101·2±5·3	125·0±4·1	123·8±4·3
Day 11	101·4±6·8	96·0±2·0	95·6±5·7	98·2±5·5	87·8±6·3	—

TABLE 2. Coefficients of variation (CV) for the five readings on each site (%) and mean CV for each time point (s.d. in parenthesis)

	Subject 1		Subject 2		Subject 3		
	L	R	L	R	L	R	Mean
Observer A							
Day 6	4·6	4·7	3·1	4·5	4·6	5·4	4·5 (0·75)
Day 7	4·2	4·9	4·0	5·1	3·3	3·5	4·2 (0·72)
Day 11	6·1	5·3	6·1	5·7	5·4	—	5·7 (0·37)
Observer B							
Day 6	1·8	6·1	4·9	4·2	4·0	5·3	4·4 (1·47)
Day 7	8·5	6·7	4·0	5·2	3·3	3·5	5·2 (2·05)
Day 11	6·7	2·1	6·0	5·6	7·2	—	5·5 (2·00)

values indicate that there was little variation between the readings of one observer for any one site. The coefficients of variation for the intra-observer error and the means of the coefficients of variation for the inter-observer error differences were between 4% and 6% (Table 2).

Comparison of comparator and photographic techniques

An example of serial comparator readings (one observer) over a 15-day period is seen in Figure 2. Table 3 contains the disappearance (extinction times) for the ten subjects investigated from data obtained with the comparator by two observers and by the photographic technique. Although in subject 1 there was a large difference between the two methods employed, this was not the case for most of the subjects. The group means did not differ by more than 1·7 days, and this difference was not statistically significant.

Effects of mechanical trauma and tape stripping

The decline in comparator readings for fluorescence from control traumatized and stripped skin

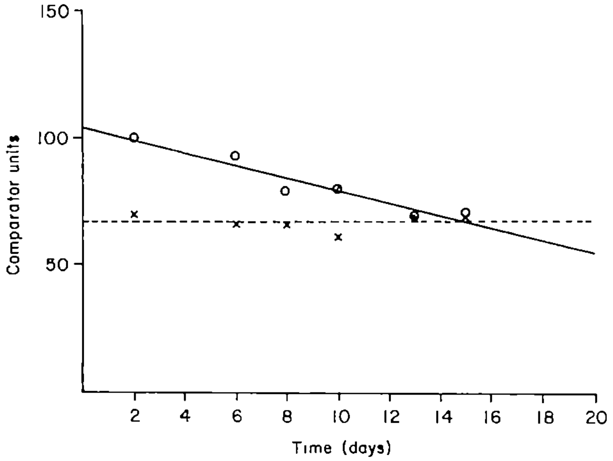

FIGURE 2. Decline of fluorescence at the forearm site of female subject age 28 years. Test (O——O); background (× -- ×).

TABLE 3. Results of experiment to compare the comparator method (with two observers) with a photographic densitometric technique

Female subject number	Age (years)	Disappearance time (days)		
		Comparator technique		
		Observer 1	Observer 2	Photographic technique
1	28	15·4	15·0	10·6
2	41	15·8	11·2	12·6
3	38	18·7	16·0	14·4
4	46	12·9	15·1	12·6
5	20	12·4	12·5	12·8
6	42	15·9	18·7	14·8
7	44	12·8	13·1	13·0
8	46	15·0	12·8	14·9
9	45	14·3	13·2	12·0
10	33	16·3	12·2	14·2
Mean ± s.d.		14·9 ± 1·8	14·0 ± 2·1	13·2 ± 1·3

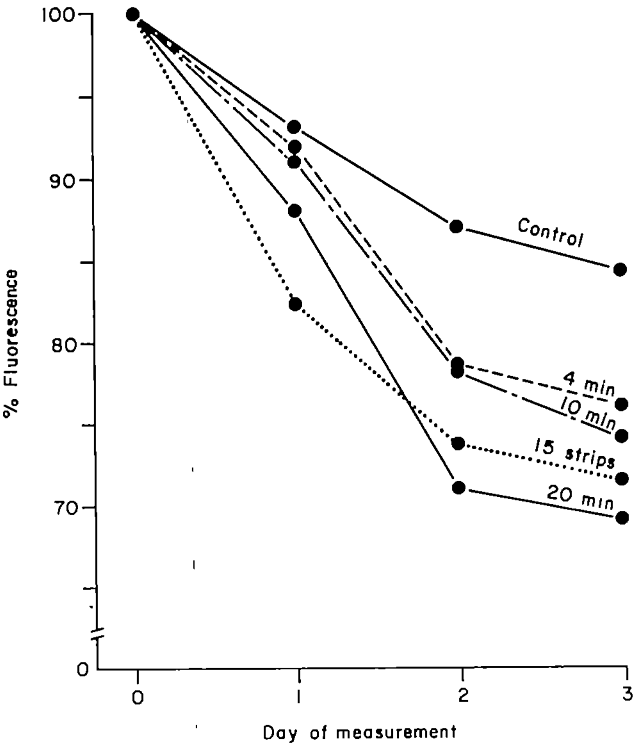

FIGURE 3. Graph to show decline of fluorescence at mechanically abraded sites and at a site stripped with adhesive tape, compared with a further control site, from 3 days of measurement with the comparator.

sites are seen in Figure 3 for the 3-day period of observation. All traumatized and stripped sites cleared the fluorescence more quickly than the control sites. The stimulus of fifteen tape strips was between that of 20 and 10 minutes of abrasive trauma as far as clearance of DC-induced fluorescence was concerned.

DISCUSSION

Most of the available techniques for assessment of epidermal cell renewal are too cumbersome and inconvenient to be clinically useful. The incorporation of tritiated thymidine by epidermal cells in DNA synthesis may use either an autoradiographic or a biochemical indicator system. Both are 'invasive', time-consuming and expensive. Stathmokinetic methods are also invasive and time-consuming, and may not be applicable to all states of epidermal cell renewal. Methods that depend on assessment of the rate of desquamation have the advantage of being non-invasive and simple. They provide a faithful estimate of epidermopoiesis providing that the parallelism between epidermal cell production and desquamation is maintained. As transient disturbances to the skin surface may affect this parallelism, estimates of the rate of corneocyte shedding at one time point may give a false view of the state of cell production. For this reason techniques which measure stratum corneum renewal over a period are likely to provide more reliable estimates.

Of these techniques, the dansyl chloride fluorescence test has proved the most useful. As originally described, the fluorescence was followed to the point of extinction. This has the disadvantage of requiring frequent examination over a period of 10 to 21 days. In addition the end-point can be difficult to determine as the hair follicles may continue to fluoresce after the interfollicular fluorescing stratum corneum has all been shed. For these reasons we devised a quantitative photographic method (Finlay, Marshall & Marks, 1982) which could shorten the period of examination and remove the subjectivity from the investigation. Despite its theoretical advantages this technique is complex and clearly not useful for routine clinical use. We believe that the comparator method described in this paper retains the theoretical advantages and surmounts the practical difficulties of repeated photography. The reliability of the comparator as judged by our estimates of the intra- and inter-observer errors is sufficient for most clinical uses whether they be directed to assessment of epidermal cell renewal or evaluation of a keratolytic agent. The experiments in which epidermopoiesis was increased by graded abrasive trauma and by skin stripping gives support to this suggestion.

The prototype design of this comparator uses a straight optical wedge. A more compact instrument can be designed around an annular wedge or by using the 'opposed wedge' principle employed in the design of photometric comparators such as the D.E.I. instrument (Dunn & Plant, 1945). Further improvement is envisaged by the incorporation of an integral source of UV radiation.

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DNA flow cytometry of the epidermis of patients with multiple epidermal carcinomas

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SUMMARY

We used DNA flow cytometry to determine whether the uninvolved skin of twenty-four patients with multiple epidermal cancers differed from normal skin with regard to its epidermal cell kinetics. We also studied the relationship between the cell kinetics and the type of cancer, previous exposure to ionizing or UV radiation, previous arsenic intake, skin type and coexisting psoriasis. Both exposed and unexposed skin was studied. In thirteen patients the S and G_2M fractions of a monoclonal tumour were compared with the values in unexposed and exposed skin. The S and G_2M fractions increased progressively from unexposed skin to exposed skin to tumour tissue. Patients who had developed both squamous and basal cell carcinoma differed from those who had developed only basal cell carcinoma by having a significantly higher G_2M fraction in the exposed skin, and significantly higher S and G_2M fractions in the unexposed skin. Patients exposed to ionizing radiation tended to show higher G_2M fractions in exposed skin compared with the remaining individuals ($P = 0{\cdot}06$). The presence of psoriasis, exposure to UVR or sunlight and previous arsenic ingestion did not appear to affect the results.

For normal human skin, reproducible cell proliferative characteristics can be obtained by flow cytometric DNA analysis of single nuclear suspensions prepared from the epidermis (Møller, Larsen & Frentz, 1978; Møller & Larsen, 1979; Frentz, Møller & Christensen, 1980). Body site and preceding biopsy trauma significantly influence the results, but sex, age, and time of day are less important (Frentz, Møller & Keiding, 1982; Frentz & Møller, 1983a). In the unaffected skin of one patient with actinic damage and a large number of different premalignant and malignant epidermal tumours we found the fraction of cells in S phase was increased (Frentz & Møller, 1981). The present study was designed to clarify whether the cell proliferation in the unaffected skin of patients with multiple epidermal neoplasms differs from that in a control group, and to evaluate the influence on epidermal cell kinetics of psoriasis, intolerance to sunlight and

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previous exposure to internal or external carcinogens such as arsenic, sunlight, artificial UVR, Grenz rays and X-rays.

METHODS

Twenty-four individuals (nine females aged 53–84 years, mean 70; fifteen males aged 47–81 years, mean 60) with epidermal neoplasms were studied. The relevant clinical data are given in Tables 1 and 2. Informed consent was obtained from all patients before biopsy. All biopsies were taken between 8 and 11.30 a.m. None of the skin areas sampled had recently been subjected to topical treatment, and none of the individuals suffered from endocrine disorders, or severe inflammatory or degenerative disease. None had received systemic medication known to influence the cell cycle. For each individual we recorded (1) presence of psoriasis, (2) arsenic intake, (3) intolerance to sunlight i.e. skin types I and II, (4) excessive exposure to sunlight and/or artificial UVR, (5) expsoure to ionizing radiation i.e. Grenz rays and/or X-rays.

Each individual was also classified according to the type of tumours removed: (1) basal cell carcinomas only (BCC group) (2) Bowen's disease only (BO), (3) both basal and squamous cell carcinomas (BCC + SCC group).

Thirteen patients provided samples from exposed skin, unexposed skin and a monoclonal tumour (Frentz & Møller, 1983b). These matched observations were used to compare the proliferative characteristics in uninvolved and involved skin in the same individual.

Biopsies

The uninvolved skin was removed by a standard procedure: local anesthesia (Mepivacaine® 2%, 1 ml without adrenaline) was given into the lower dermis and an elliptical biopsy involving dermis and epidermis measuring approximately $\frac{1}{2} \times 1\frac{1}{2}$ cm was removed. The involved skin was removed by curettage (Frentz & Møller, 1983b).

Uninvolved skin: (a) twenty-one of the twenty-four individuals provided biopsies from skin which had not been exposed previously to external carcinogens (e.g. from the lower abdominal region). This skin category is henceforward called unexposed skin; (b) nineteen of the twenty-four individuals provided samples of uninvolved skin from either perilesional skin, [i.e. skin displaced 2–5 cm from the edge of the tumour ($n=8$)] or from skin which was not close to tumour sites, but which had earlier been exposed to external carcinogens such as UVR or ionizing radiation ($n=11$). Whenever feasible these samples were taken from the extensor surface of the forearm. These nineteen samples were pooled under the designation exposed skin, since the S and G_2M values for these two groups were not significantly different.

As control material we used normal lower abdominal skin from a group of twenty ulcer patients aged 36–87 years without epidermal malignancies (Frentz & Møller, 1983a).

Flow cytometry and statistical analysis

The maceration, storage and preparation of the tissue samples before flow cytometry has been previously described (Møller & Larsen, 1979; Frentz *et al.*, 1980). The DNA content of the individual nuclei in the monodisperse suspension was measured in the flow cytometer (Cytofluorograph, Bio Physics System Inc., type 4802A and 6300A).

The biopsies from one person were handled in the same assay for optimal comparability. The stability of the fluorescence signals was checked by a run of standard samples consisting of Yoshida ascites tumour cell suspensions, trout erythrocytes and/or polystyrene standard microspheres before and after the skin sample measurements. Assuming a constant rate of DNA synthesis through the S phase the DNA frequency distribution for each sample was statistically analysed using a least-squares estimation procedure (Frentz *et al.*, 1980).

Analysis of variances and *t*-tests of the virtual or logarithmic values were used following evaluation of whether the distribution of results could be regarded as normal and whether the results in the different groups were comparable by *F*-tests.

For the thirteen patients who provided biopsies from unexposed, exposed, and neoplastic skin, a two-way analysis of variance was carried out on the logarithmic values to evaluate whether the differences were due to individual factors or to differences depending on the category of skin studied. For evaluation of the correlation between the uninvolved skin and the tumour type, pre-existing conditions and carcinogen exposure, analyses of variance were carried out using various models depending on the importance of the different variables (Frentz *et al.*, 1982). The 5% probability level was used.

RESULTS

The relationship between the S and G_2M fractions in unexposed, exposed and neoplastic epidermis for thirteen patients is shown in Figure 1. The level of the S and G_2M fractions was strongly related to the skin category studied ('unexposed' versus 'exposed' versus 'tumour'), but individual factors were also important. On average the S fraction increased significantly by a factor of 1·5 from the unexposed to the exposed skin, and by 2·1 from the unexposed skin to

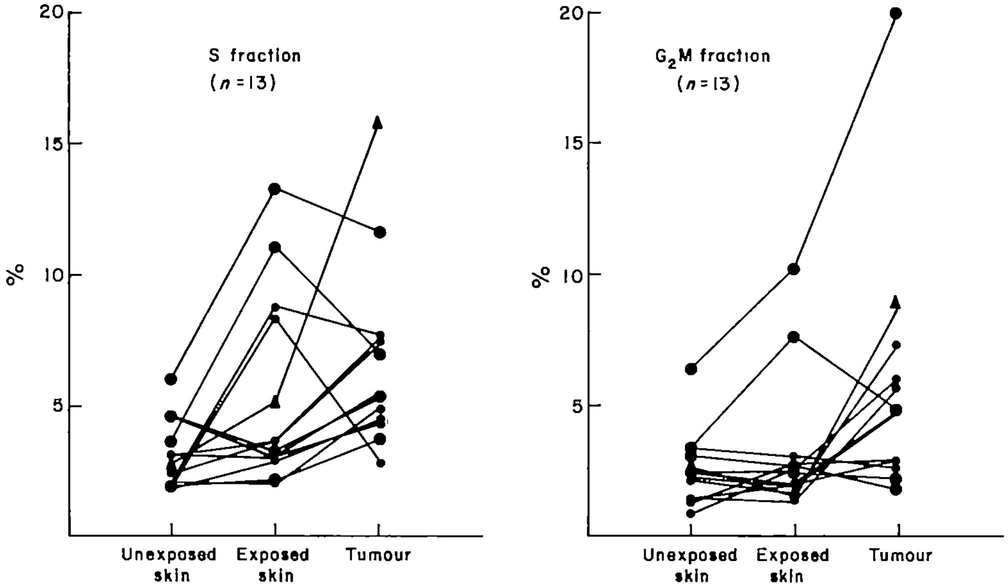

FIGURE 1. The S and G_2M fractions from thirteen individuals with multiple epidermal cancers. (●) Patients who have developed at least one SCC; (▲) patients with multiple BO; the remaining patients have multiple BCC exclusively. The average S and G_2M fractions increase stepwise from the unexposed skin through the exposed skin to the tumour. A definite increase in the S fraction occurred from the unexposed to the exposed skin, and in the G_2M fraction from the exposed skin to the tumour ($P < 0{\cdot}01$). The levels of the S and G_2M values depend heavily on the type of skin (unexposed, exposed or tumour) even if a personal factor also plays a role ($P < 0{\cdot}0005$ and $< 0{\cdot}002$ for log S and log G_2M for skin type differences in the single individual; $P < 0{\cdot}02$ and $0{\cdot}015$ for individual differences in log S and log G_2M by analyses of variance).

tumour. The average G_2M fraction increased insignificantly by 1·2 from the unexposed to the exposed skin and significantly by 2·0 from the unexposed skin to the tumour.

For the uninvolved skin for the total number of individuals the S and G_2M level apparently reflected the tumour type(s) developed in the patients. Both the S and G_2M fractions in the unexposed skin (Fig. 2, Table 1) and the G_2M fraction in the exposed skin were significantly

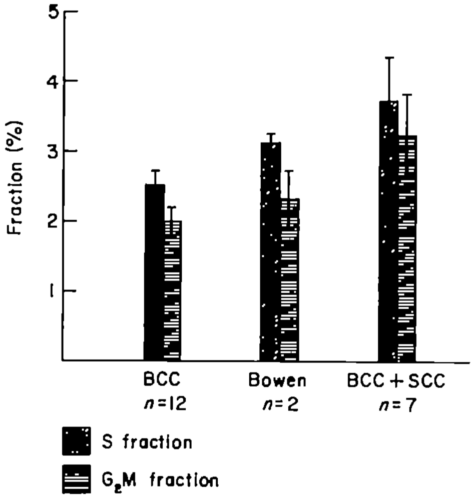

FIGURE 2. The S and G_2M fraction values in the unexposed skin from twenty-one multiple epidermal cancer patients related to tumour type developed in each patient. Vertical bars indicate s.e.m. In spite of the wide variability in the BCC+SCC group, the BCC and BCC+SCC groups differed significantly in both the S and G_2M fractions in the unexposed skin ($P < 0·05$ for both). In the statistical calculations the figures from the BO patients are omitted because of the small number.

TABLE 1. Flow cytometric data (%mean ± s.d.) on the unaffected skin form twenty-four individuals with multiple epidermal neoplasms related to the type of tumours developed

Type of tumours developed	Unexposed skin S	Unexposed skin G_2M	Unexposed skin No. of samples	Exposed/perilesional skin S	Exposed/perilesional skin G_2M	Exposed/perilesional skin No. of samples
BCC (15 patients)	2·5±0·8[a]	2·0±0·8[b]	12	3·9±2·2	2 2±0·7[c]	13
Bowen's disease (2 patients)	3·1±0·1	2·3±0·6	2	5·2	1 6	1
BCC+SCC (7 patients)	3 7±1·5[a]	3·2±1·6[b]	7	6 6±5·1	5·3±2·2[c]	5
Total (24 patients)	2·9±1 1	2·4±1·2	21	4·7±3 3	3 0±2·2	19
Control group (20 patients)	2·6±0·8	2·1±1·1	20			

[a], [b] $P < 0·05$.
[c] $P < 0 005$.

higher in individuals developing both BCC and SCC than in those developing BCC alone ($P \simeq 0{\cdot}004$ for G_2M fractions in the exposed skin).

With regard to the possible influence of carcinogen exposure and pre-existing conditions (Tables 2 and 3) the exposed skin from individuals damaged by X-rays/Grenz rays tended to show higher G_2M fractions than the remaining patients ($P = 0{\cdot}06$, when allowance is made for the influence of tumour type).

For the total group of twenty-four patients (Table 3) the unexposed skin did not differ statistically from lower abdominal skin of a group of twenty leg ulcer patients (Tables 1 and 2; Frentz & Møller, 1983a).

DISCUSSION

In the present study of skin cancer patients, the exposed skin, in spite of the wide range of results, showed significantly higher S fraction than the unexposed skin (mean values 4·7 and 2·9 respectively). The main difference between the exposed skin and tumour was however seen in the G_2M fraction (for variability in curetted tumours see Frentz & Møller, 1983b). These

TABLE 2. Pre-existing conditions and carcinogen exposure in twenty-four multiple epidermal carcinoma patients related to flow cytometry values in unexposed and exposed or perilesional skin (%mean ± s.d.)

		Unexposed skin			Exposed/perilesional skin		
Preexisting conditions/ exposure	No. of individuals	S	G_2M	No. of samples	S	G_2M	No. of samples
Intolerance to sunlight	8	2·8 ± 1·0	2·3 ± 0·7	7	2·5 ± 0·7	2·1 ± 1·1	4
Previous arsenic intake	13	3·2 ± 1·3	2·4 ± 0·8	12	4·9 ± 3·8	3·1 ± 1·8	9
X-rays/Grenz rays	13	3·2 ± 1·4	2·5 ± 0·8	11	4·2 ± 2·9	2·8 ± 1·6	13
Extensive sunbathing, exposure to UVR	13	3·3 ± 1·4	2·3 ± 0·8	12	4·6 ± 3·4	2·8 ± 1·7	11
Psoriasis	8	3·6 ± 1·5	2·5 ± 0·8	7	4·6 ± 3·6	3·1 ± 1·9	8
Total	24	2·9 ± 1·1	2·4 ± 1·2	21	4·7 ± 3·3	3·0 ± 2·2	13
Control group	20	2·6 ± 0·8	2·1 ± 1·1	20			

TABLE 3. The distribution of pre-existing conditions/exposure among twenty-four multiple epidermal carcinoma patients related to the type of tumours developed

Preexisting conditions/ exposure	BCC ($n = 15$)	BO ($n = 2$)	BCC + SCC ($n = 7$)	No. of individuals ($n = 24$)
Intolerance to sunlight	5	1	2	8
Previous arsenic intake	6	2	5	13
X-rays/Grenz rays	8	1	4	13
Extensive sunbathing, exposure to UVR	9	0	4	13
Psoriasis	5	0	3	8

striking findings in the proliferative characteristics are in accordance with the labelling index studies of individuals developing actinic keratoses (Pearse & Marks, 1977) and Feulgen photometry studies (Kint, 1963). Regional differences between normal or unexposed and exposed skin might also play a role (Bauer *et al.*, 1980; Frentz & Møller, 1983a). From Figure 1 it is obvious that the statistical significance mainly depends on pronounced oscillations in the values for some patients. This suggests that incorporation of individual clinical features might provide a more explicit formulation of the variability. The occurrence of SCC was found to be related to high S and G_2M fractions in the uninvolved skin (Table 1, Fig. 2). None of the carcinogens or pre-existing conditions listed in Table 2 could be unequivocally correlated with the S or G_2M fraction levels when the influence of the tumour type was incorporated, even if X-ray or Grenz ray damage tended to elevate the G_2M fraction in the exposed skin. Thus we were unable to support the recent findings on actinic skin damage by Gregg and Mansbridge (1982) of higher *in vitro* labelling indices in the unexposed skin of actinic skin cancer patients compared with individuals without skin cancer and patients with presumed arsenic-induced skin cancer.

The above-mentioned findings stimulate several questions. Do high proliferative characteristics in general reflect virtual or potential malignancy, and does the level of the values correlate with the degree of malignancy in terms of aggression and bad prognosis?

Controversy exists with regard to the meaning of high proliferative characteristics in tissue from solid human tumours and lymphomas. No safe diagnostic value has so far been settled, but it is likely that a positive correlation does exist between high S fractions and a bad prognosis. Furthermore the size of the S fraction may have therapeutic implications (Barlogie *et al.*, 1983).

Regarding non-neoplastic human skin it is highly unlikely that the well-established high proliferative activity in uninvolved psoriatic epidermis (Braun-Falco, Christophers & Kurban, 1967; Harper, Rispler & Urbanek, 1978; Bauer *et al.*, 1981) indicates potential malignant degeneration. Even if psoriatics heavily exposed to tar and artificial UVR are inclined to develop epidermal neoplasms (Stern, Zierler & Parrish, 1980), psoriasis in itself is not regarded as predisposing to epidermal cancers (Shuster, Chapman & Rawlins, 1979).

Future studies may throw light on the prospective value of monitoring skin damage by means of cell kinetic studies, but very comprehensive studies on a large number of individuals will be needed for final clarification.

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Inducibility of arylhydrocarbon-hydroxylase activity in human hair follicles by topical application of liquor carbonis detergens (coal tar)

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SUMMARY

Arylhydrocarbon-hydroxylase (AHH) is a cytochrome P-450-dependent polysubstrate mono-oxygenase which plays an important role in converting some compounds (e.g. benzo[*a*]pyrene) to highly reactive carcinogenic species. A simple AHH assay is described, using [^{3}H]benzo[*a*]-pyrene as substrate. 7,8-Benzoflavone (10^{-4} M) inhibits 92% of the measured enzyme activity. Liquor carbonis detergens (which contains coal tar) induces AHH activity in human hair follicles *in vivo*. We suggest that using this simple assay, hair follicles would be a very suitable tissue to test whether the AHH-controlling gene is of significance in producing cancer.

The skin is a portal of entry for various chemicals and it can metabolize drugs and other foreign compounds (Bickers, 1983). Benzo[*a*]pyrene is carcinogenic only after metabolic transformation, and it is important that the skin contains measurable hydroxylase activity for this carcinogenic substrate (Briggs & Briggs, 1973; Bickers & Kappas, 1978). This benzo[*a*]pyrene hydroxylase activity, which is cytochrome P-450-dependent, is called arylhydrocarbon-hydroxylase (AHH) activity. It is an inducible polysubstrate mono-oxygenase which reflects genetic differences at the arylhydrocarbon (Ah) locus (Nebert & Jensen, 1979). It is assumed that the AHH activity-controlling gene may be of significance in chemical carcinogenesis (Nebert, 1980). Cancer of the skin is the most common form of human malignant neoplasm. AHH activity possesses species and organ specificity. Therefore, it is of interest to determine the activity of this enzyme in a convenient source of human epithelial tissue.

Recently cytochrome P-450-dependent enzyme activity and benzo[*a*]pyrene metabolism were detected in human hair follicles, using thin-layer chromatography and high-pressure

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liquid chromatography (Vermorken *et al.*, 1979a,b; Hukkelhoven, Dijkstra & Vermorken, 1983). However, in cultured human hair follicles no AHH inducibility was detected (Hukkelhoven *et al.*, 1981a).

In this study, we have developed a simple assay to determine AHH activity in human hair follicles and we have assessed the AHH inducibility in human hair follicles *in vivo*. As inducing substrate we used liquor carbonis detergens (LCD), which contains coal tar, and is widely used in dermatological practice.

METHODS

Collection of tissue

Human hairs were plucked from the occipital area of the scalp of healthy volunteers and patients who were not suffering from malignancy, infection or autoimmune disease. They had taken no drugs or UV-therapy and had received no topical treatment with tar, and no treatment for the scalp. Only anagen hair bulbs were used for the assay.

In order to study AHH inducibility, we determined AHH activity in hair bulbs of ten patients with psoriasis before and after topical application of 5% LCD in petrolatum on the scalp over a period of 5 days.

Chemicals

[G-^{3}H]benzo[*a*]pyrene (specific activity 19 Ci/nmol) was purchased from the Radiochemical Centre, Amersham. Isocitrate dehydrogenase, NADP and isocitrate were obtained from Boehringer, Mannheim. Dimethylsulfoxide (DMSO) and *n*-hexane were purchased from Merck, Darmstadt.

AHH assay

We incubated ten or twenty anagen hair bulbs as previously described (500 μl final incubation volume, pH 8·5) (Dehnen, Tomingas & Roos, 1973). The reaction was started by adding 10 pmol[^{3}H]benzo[*a*]pyrene dissolved in ethanol (ca. 200,000 c.p.m./assay) and was stopped by the addition of 1 ml KOH (0·15 M in 85% DMSO) after 120 minutes. After incubation the organic phase was removed by two successive extractions in 5 ml hexane (von Cantfort, de Graeve & Gielen, 1977). The radioactivity of the water-soluble benzo[*a*]pyrene metabolites was measured in a scintillation counter and the AHH activity was expressed as counts per minute (c.p.m.) water-soluble (ws) benzo[*a*]pyrene (BP) per minute per bulb.

Under these conditions the assay was linear with respect to the number of the hair bulbs and the time of incubation.

RESULTS

Figure 1 summarizes the measured AHH activity in the hair bulbs of eighty-one subjects. There is a large variation, comparable to that found in human placentas and lymphocytes (Nebert, 1980). Measuring the AHH activity in the hair bulbs of the same subjects in ten assays, we found a variation of only 4·2%.

There was a marked dose-dependent inhibition of the measured AHH activity by 7,8-benzoflavone. However, there was no inhibition of the measured AHH activity by SKF 525-A, metyrapone and tetrahydrofurane (Fig. 2). We found no difference between smokers and

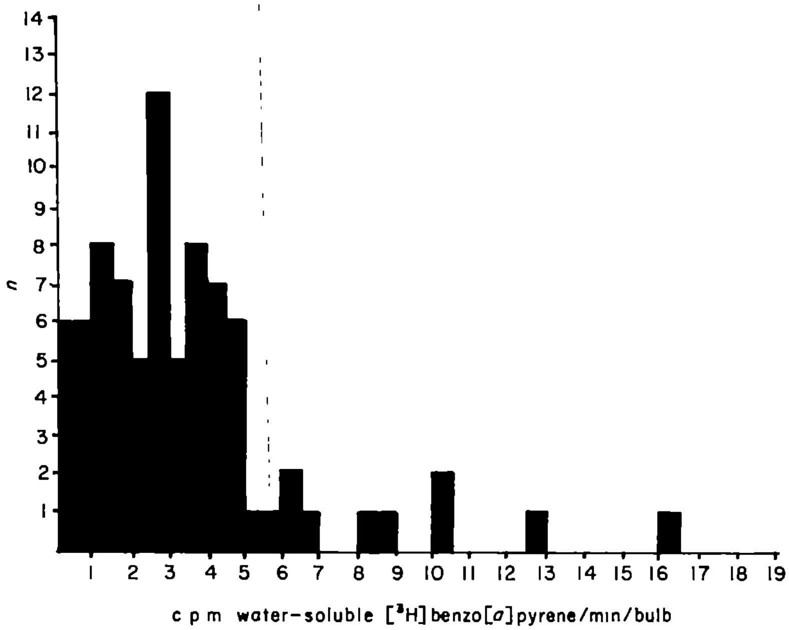

FIGURE 1. The AHH activity in the hair follicles of eighty-one subjects.

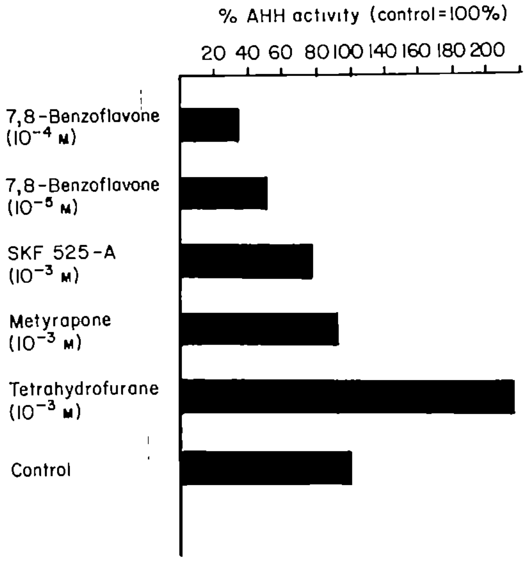

FIGURE 2. The AHH activity in hair follicles, showing a marked inhibition of this activity by 7,8-benzoflavone, but no inhibition by SKF 525-A, tetrahydrofurane or metyrapone

non-smokers (3·0 ± 1·9 and 4·3 ± 3·9 respectively) or between females and males (3·61 ± 3·91 and 3·70 ± 2·23 respectively).

In eight of ten patients we observed an increase of the measured AHH activity after topical applications of liquor carbonis detergens (Fig. 3).

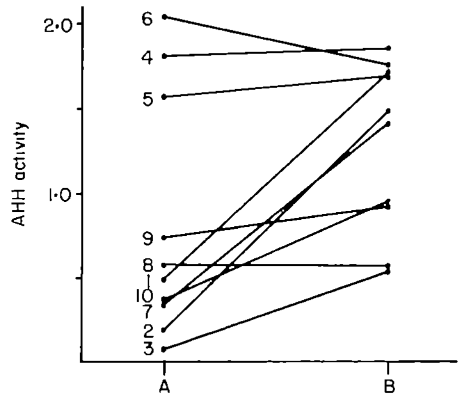

FIGURE 3. AHH activity (c.p.m. ws[^{3}H]BP min^{-1} bulb^{-1}) in hair bulbs of ten psoriatics, (A) before and (B) after topical application of liquor carbonis detergens to the scalp for 5 days.

DISCUSSION

Our results show that it is possible to measure AHH activity in human hair bulbs using a radiometric assay.

We expressed the amount of water-soluble benzo[*a*]pyrene metabolites in relation to the number of hair bulbs because it is not advisable to relate the enzyme activity to the protein content because a part of the follicle consists of differentiated cells containing most of the proteins whereas the majority of the enzymatic activity is located in the cells which are not yet fully differentiated (Vermorken *et al.*, 1981). However, there is a close relation between the number of the hair bulbs and the DNA content of hair bulbs (Hukkelhoven *et al.*, 1981b). Therefore we decided to express the detected AHH activity in relation to the number of hair bulbs. By this method we found an intra-individual variation of only 4·2%.

We found a marked inhibition of the measured AHH activity by 7,8-benzoflavone (92%), indicating that the detected AHH activity is Ah-locus dependent (Nebert, 1978). There was no inhibition by metyrapone, SKF 525-A and terahydrofurane, indicating that AHH activity in human hair follicles is primarily mediated by cytochrome P-450 isoenzymes which are inducible by polyaromatic hydrocarbons (e.g. benzo[*a*]pyrene) and not by phenobarbital or alcohol (Ullrich, Frommer & Weber, 1973; Ullrich, Weber & Wollenberg, 1975).

There was no difference of the AHH activity due to smoking habits. Thus the AHH activity of hair bulbs probably does not depend on the influence of systemic chemicals such as polyaromatic hydrocarbons. This finding is in line with the observation in twin studies that genetic factors account for most of the total inter-individual variations in basal AHH activity of hair bulbs, and not only AHH inducibility (Hukkelhoven *et al.*, 1982).

Previous workers have found no inducibility of AHH activity in cultures of keratinocytes of human hair follicles (Hukkelhoven *et al.*, 1981a). However, it has become clear that a lack of reproducibility of AHH induction in cultured tissues is a major source of difficulty (Fletcher, Price Evans & Canning, 1978; Finnen *et al.*, 1983). These observations prompted us to investigate whether there is inducibility of AHH activity in hair bulbs *in vivo*. As inducing substrate we used liquor carbonis detergens, a solution of coal tar which is known to induce cutaneous AHH activity (Bickers & Kappas, 1978). In six out of ten patients there was a marked increase of AHH induction after liquor carbonis detergens treatment. In four patients we observed no enhancement of AHH activity. This lack of inducibility was probably due to induction by other unknown substrates before treatment because three of the four patients had the highest basal AHH activity.

It has been shown that human hair follicles metabolize benzo[*a*]pyrene in a similar way to human bronchial epithelial cells (Hukkelhoven, Dijkstra & Vermorken, 1983). Therefore hair bulbs are a very suitable tissue to investigate whether the AHH-controlling gene is of significance in producing human cancer. Our simple assay makes it possible to test this hypothesis by measuring the basic and induced AHH activity in a large population.

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HLA-DR antigen expression on the keratinocyte surface in dermatoses characterized by lymphocytic exocytosis (e.g. pityriasis rosea)

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SUMMARY

We have investigated the immunoperoxidase staining pattern of the epidermis in several dermatoses characterized by exocytosis of mononuclear cells into the epidermis. We found that HLA-DR antigens showed an intercellular distribution in localized areas of the epidermis in nine of ten cases of pityriasis rosea, and in all four cases of spontaneously regressing flat warts, two cases of pityriasis lichenoides chronica, two of Schamberg's disease, and one case of lichen striatus. Lichen planus and mycosis fungoides cases were used as positive controls. OKT6 antigen was recognized only on the dendritic cells of the epidermis in all these cases. Judging from the distribution of Langerhans cells, the epidermal intercellular HLA-DR antigen seems to be expressed on the keratinocytes in such diseases, and this feature was confirmed by immunoelectron microscopy. These findings support the hypothesis that the expression of HLA-DR antigen on keratinocytes in these dermatoses is linked to cellular immune reactions involving the epidermis.

Mononuclear cells migrate into the epidermis in certain diseases such as contact dermatitis and spontaneously regressing flat warts, in which a cellular immune reaction occurs in the epidermis (Tagami *et al.*, 1974). However, in several other dermatoses also characterized by exocytosis of mononuclear cells into the epidermis, the exact mechanisms involved are still unclear, e.g. lichen planus, pityriasis rosea, Schamberg's disease, pityriasis lichenoides chronica, and lichen striatus.

It was once thought that Ia antigens are restricted to the surface of B lymphocytes, endothelia and some macrophages (McDevit *et al.*, 1976), but it has become clear that the distribution, although limited, is wider than was formerly thought, being present in the thymic epithelium (Van Ewijk, Rouse & Wrissmann, 1980), activated T lymphocytes (Ko *et al.*, 1979), and normal epithelial cells of the kidney, gut, bronchi and mammary gland (Wiman, Curman & Forsum,

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1978; Hirschberg, Bergh & Thorsby, 1980; Klareskog, Forsum & Peterson, 1980; Natali, DeMartino & Quaranta, 1981). In the normal epidermis, the expression of Ia antigen is limited to Langerhans cells (Stingl, Tamaki & Katz, 1980). However, it has now been demonstrated that keratinocytes can also express Ia antigens in several dermatoses such as mycosis fungoides (Malmnas-Tjernlund, 1978; MacKie & Turbitt, 1983), psoriasis (Morhenn, Abel & Mahrle, 1982), lichen planus (Malmnas-Tjernlund, 1980), graft-versus-host disease (Lampert, Suitters & Chisholm, 1981; Mason, Dallman & Barclay, 1981), and contact dermatitis (MacKie & Turbitt, 1983). The role of such Ia antigen expression on the keratinocyte surface is still unclear. Suitters and Lampert (1982) observed Ia antigen expression on keratinocytes in rats with contact hypersensitivity, suggesting that the expression of Ia antigen on keratinocytes is linked to cellular immunity involving the epidermis.

By comparing the distribution of Ia (HLA-DR) antigen to that of OKT6 antigen which is a better marker than HLA-DR antigen for Langerhans cells (Harrist *et al.*, 1983), we could demonstrate the expression of Ia (HLA-DR) antigen on the keratinocyte surface in several other cutaneous disorders that are characterized by exocytosis of mononuclear cells into the lesional epidermis. Furthermore, we confirmed the presence of Ia antigen on the keratinocyte surface by immunoelectron microscopic observation.

METHODS

Punch biopsy specimens, 3–4 mm in diameter, were obtained from the lesions of several cutaneous disorders characterized by exocytosis of mononuclear cells into the epidermis, i.e., pityriasis rosea (ten cases), spontaneously regressing flat warts (four cases), Schamberg's disease (two cases), pityriasis lichenoides chronica (two cases), and lichen striatus (one case). As a positive control, biopsy specimens were also taken from one case each of poikilodermatous mycosis fungoides and lichen planus. Fifteen specimens were also obtained from the lesions of dermatoses which did not show prominent exocytosis of mononuclear cells into the epidermis, viz. seven cases of psoriasis, and one case each of Jessner's lymphocytic infiltration of the skin, B-cell lymphoma, and lichen sclerosus et atrophicus, in addition to five normal skin samples. Xylocaine (1%) without adrenaline was injected around but not into the site of biopsy. Each tissue was frozen and stored at −80°C.

Immunoreagents

The following immunoreagents were used: hybridoma monoclonal antibodies OKT6 (Ortho diagnostic system Inc., Raritan, N.J., U.S.A.) and anti-Leu-1 and anti-HLA-DR (Becton Dickinson, Facs. Systems, Sunnyvale, Calif.); normal horse serum; horse biotinylated anti-mouse IgG; avidin DH; biotinylated horseradish peroxidase H (Vector Lab Inc., Burlingame, Calif.); 3,3′-diaminobenzidine tetrahydrochloride (DAB) (Dojin Lab Inc., Kengucho, Kumamoto, Japan).

Immunoperoxidase light microscopy

Six micron-thick frozen sections were air-dried, fixed in acetone for 10 min, and stained by the preformed avidin–biotin–peroxidase complex method detailed elsewhere (Hsu, Raine & Fanger, 1981; Berti *et al.*, 1983). After being blocked with normal horse serum diluted in phosphate-buffered saline (PBS) for 30 min at 37°C, sections were incubated with a 1:10 dilution of anti-OKT6 antibody or a 1:10 dilution of anti-Leu-1 antibody or a 1:40 dilution of anti-HLA-DR antibody for 30 min at 37°C. This was followed by 30-min separate incubations with biotin-conjugated horse anti-mouse IgG diluted in PBS at 37°C and the preformed avidin–biotin–peroxidase complex at 37°C. The reaction product was revealed by incubation in 0·05 M Tris-HCl buffer (pH 7·6), 0·05% DAB and 0·01% H_2O_2 for 5 min at room temperature, followed by a quick rinse in distilled water. Finally, after nuclear staining with haematoxylin, the sections were transferred from distilled water to xylene and mounted in Eukit.®

Immunoelectron microscopy

For immunoelectron microscopy, the specimens from one case each of pityriasis rosea and regressing flat wart were further cut into thin strips and fixed in periodate–lysin–paraformaldehyde (PLP) for 4 h at 4°C. According to the procedure of McClean and Nakane (1974), the post-fixed tissue strips were washed in PBS containing 7% sucrose at 4°C overnight, washed in PBS containing with O.C.T. compound for frozen tissue specimens, and quickly frozen in a mixture of acetone and solid carbon dioxide. Six-μm frozen sections were placed on egg albumin-coated slides. Immunoperoxidase staining was performed as described above, except that PBS replaced PBS with 7% sucrose. After being incubated in DAB solution, the sections were washed twice with PBS containing 7% sucrose, post-fixed with 1% osmium tetraoxide at 30 min at 4°C, dehydrated through up-grade ethanol, and replaced with QY-1 and QY-1-Quetol mixture. Gelatin capsules filled with Quetol were inverted over the tissue sections and the resin was polymerized at 60°C. The block was separated from the slides by heating over a gas burner. Ultrathin sections were studied by JEM 100B electron microscope.

RESULTS

The results of the immunoperoxidase staining are summarized in Table 1.

TABLE 1. The distribution of OKT6 and HLA-DR antigen in the epidermis

		OKT6 Ag		HLA-DR Ag
Diseases	No. of cases	Dendritic cells	Intercellular	Intercellular
Pityriasis rosea	10	10	0	9
Regressing flat warts	4	4	0	4
Schamberg's disease	2	2	0	2
Pityriasis lichenoides chronica	2	2	0	2
Lichen striatus	1	1	0	1
Lichen planus	1	1	0	1
Mycosis fungoides	1	1	0	1
Psoriasis	7	7	0	0
Jessner's lymphocytic infiltration	1	1	0	0
B-cell lymphoma	1	1	0	0
Lichen sclerosus et atrophicus	1	1	1	0
Normal skin	5	5	0	0

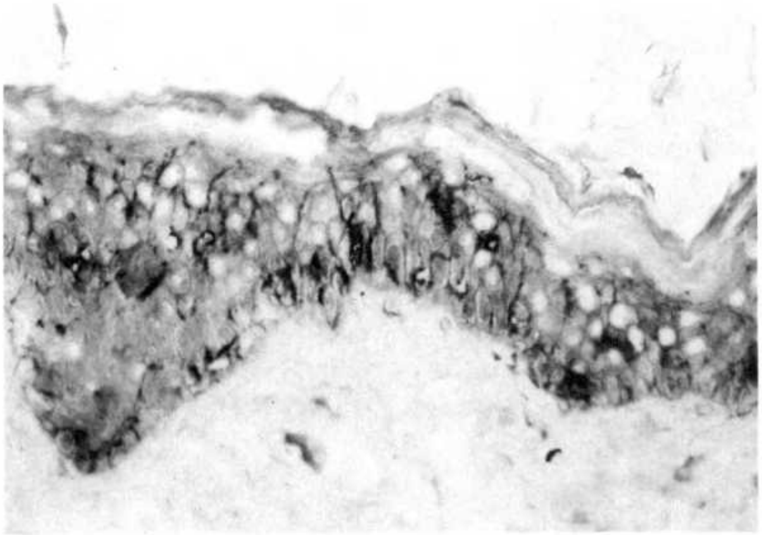

FIGURE 1. B-cell lymphoma. Langerhans cells stained by anti-OKT6 antibody are scattered in the epidermis (× 200).

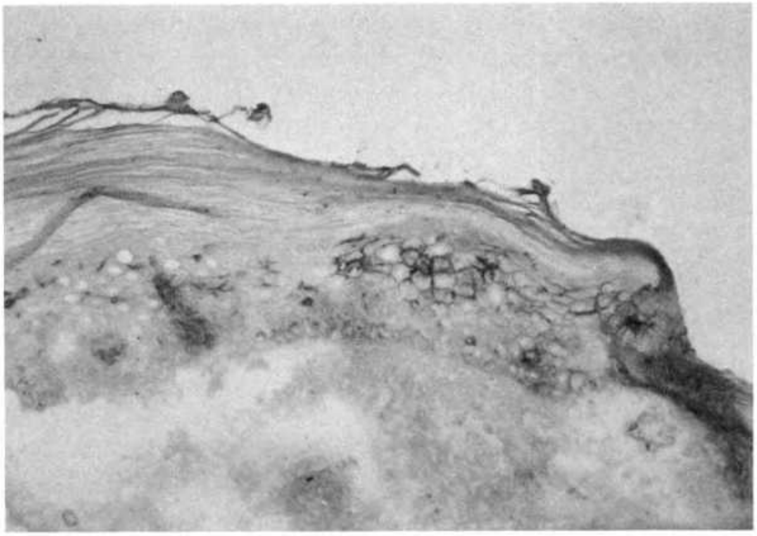

FIGURE 2. Lichen sclerosus et atrophicus. In addition to Langerhans cells, partial intercellular staining of the epidermis by anti-OKT6 antibody is seen (× 100).

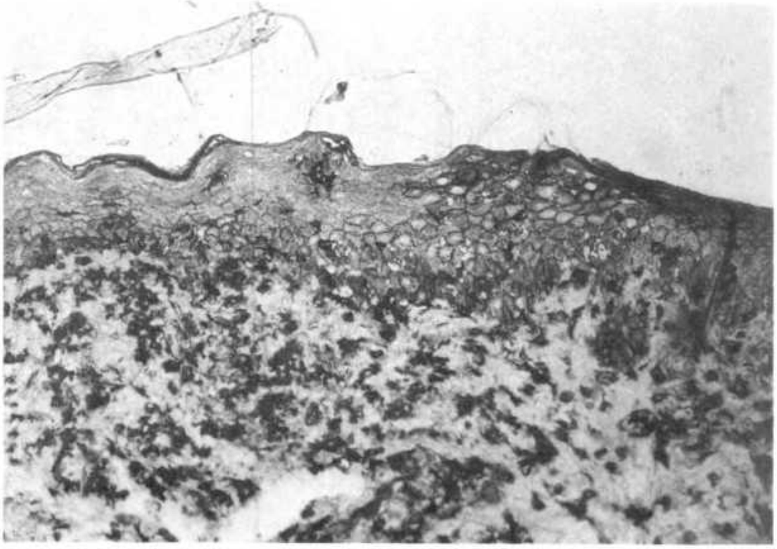

FIGURE 3. Pityriasis rosea. Intercellular staining by anti-HLA-DR antibody is recognized in a focal area of the epidermis. A massive infiltration of HLA-DR$^+$ cells in the upper dermis and exocytosis of HLA-DR$^+$ cells are noted (× 100).

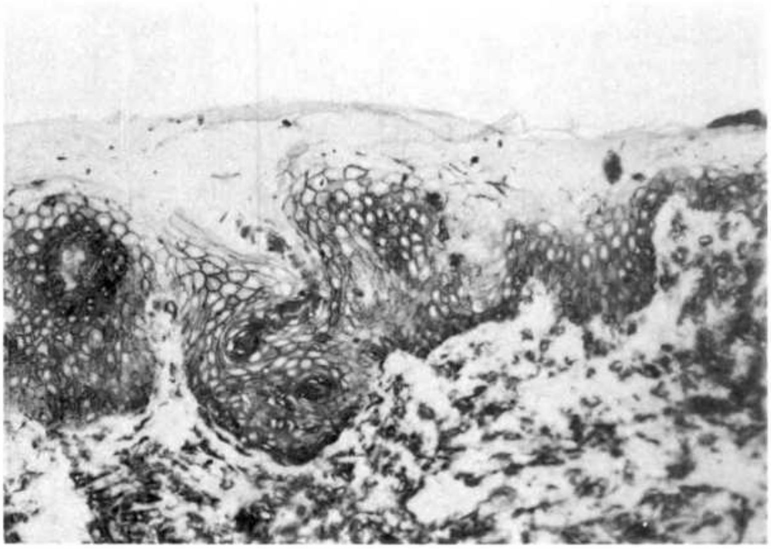

FIGURE 4. Schamberg's disease. In the lower part of the epidermis, intercellular staining by anti-OKT6 antibody is noted. In the upper dermis, a massive infiltration of HLA-DR$^+$ cells is present (× 100).

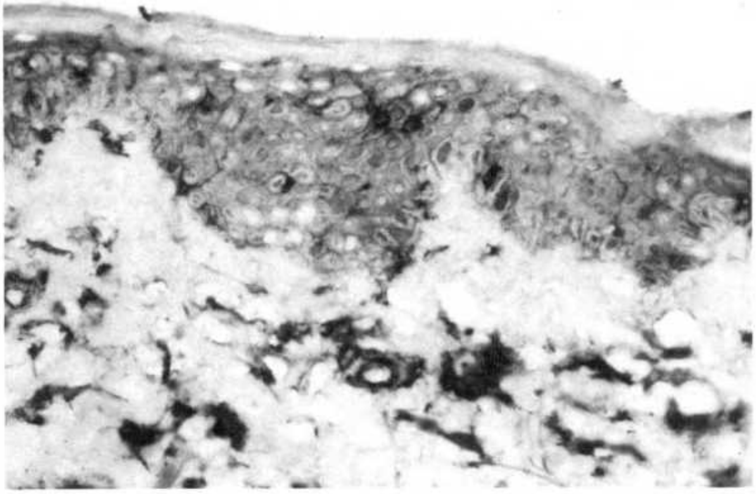

FIGURE 5. B-cell lymphoma. A smaller number of HLA-DR$^+$ dendritic cells are stained by anti-HLA-DR antibody. No intercellular staining is seen (× 200).

Immunoperoxidase staining in light microscopy

The immunoperoxidase staining with anti-OKT6 antibody demonstrated only scattered dendritic cells (Langerhans cells) of the epidermis in five normal controls and thirty other control cases (Fig. 1) except for one case of lichen sclerosus et atrophicus. A weak intercellular staining was recognized in only a small area of the epidermis in that single case (Fig. 2).

An intercellular immunostaining with anti-HLA-DR antibody was recognized in at least some areas of the epidermis in nine of ten cases of pityriasis rosea (Fig. 3), and in all the studied cases of regressing flat warts, Schamberg's disease (Fig. 4), pityriasis lichenoides chronica, and lichen striatus, in addition to those cases with lichen planus or mycosis fungoides. In the surrounding portion of the epidermis, only epidermal dendritic cells were stained in this way, as noted in control normal skin and in other cases with negative intercellular staining (Fig. 5).

The areas showing the epidermal intercellular distribution of Ia (HLA-DR) antigen were always associated with a dense HLA-DR$^+$ cellular infiltration in the upper dermis (Fig. 3). Furthermore, in pityriasis rosea, regressing flat warts and lichen striatus, active exocytosis of HLA-DR$^+$ cells was also found in the epidermis with intercellular immunostaining (Fig. 3). By immunoperoxidase staining with anti-Leu-1 antibody, most of the infiltrating HLA-DR$^+$ cells were shown to be T lymphocytes.

In none of the seven psoriatic lesions could we find any definite epidermal intercellular immunostaining with anti-HLA-DR antibody as noted above.

Immunoelectron microscopy

When stained with anti-OKT6 antibody, strong electron-dense deposits were observed only on the surface of epidermal dendritic cells in both pityriasis rosea and regressing flat warts (Fig. 6). On the other hand, electron-dense deposits were recognized on the surface of keratinocytes, dendritic cells, and infiltrating mononuclear cells when stained with anti-HLA-DR antibody (Fig. 7).

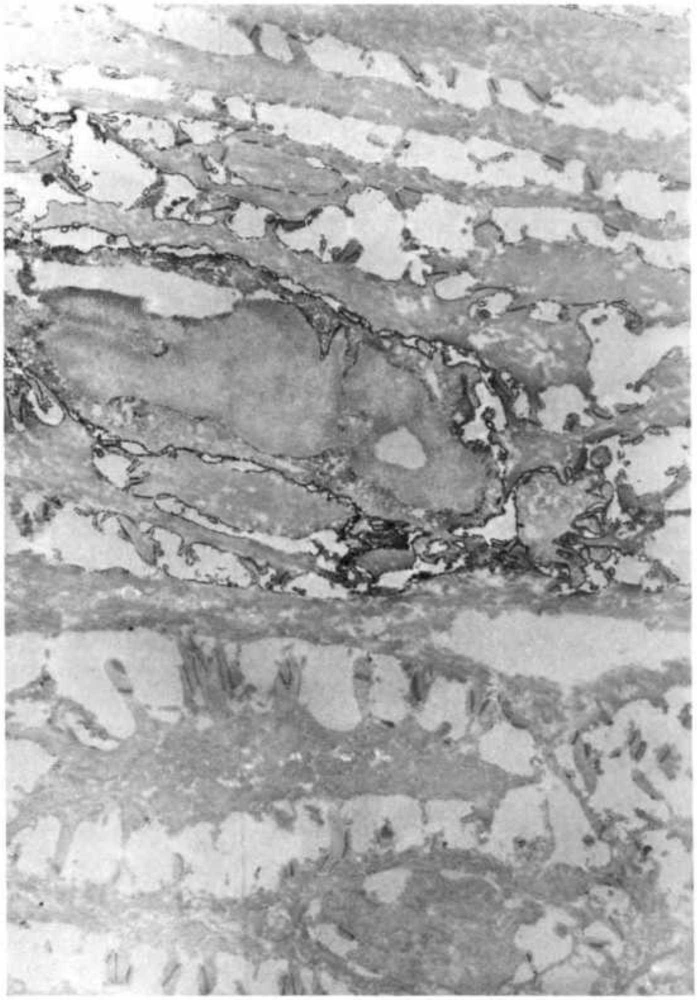

FIGURE 6. Regressing flat warts. By immunoelectron microscopy using anti-OKT6 antibody, electron-dense deposits were noted on the surface of only epidermal dendritic cells (× 8000).

DISCUSSION

In the epidermis Langerhans cells express Ia antigens. In order to investigate Ia antigen expression on the keratinocyte surface in disease states, it is important to know the distribution of Ia antigen-bearing Langerhans cells in such situations as well. These can be identified by OKT6 antigen, which is known to be a superior marker to Ia (HLA-DR) antigen for Langerhans cells in the human epidermis (Harrist *et al.*, 1983). We can assume that the expression of Ia (HLA-DR) antigen extending beyond the distribution of Langerhans cells is mainly present on the keratinocytes.

The present study showed that the epidermal intercellular distribution of Ia (HLA-DR) antigen unrelated to the distribution of Langerhans cells was recognized in the dermatoses

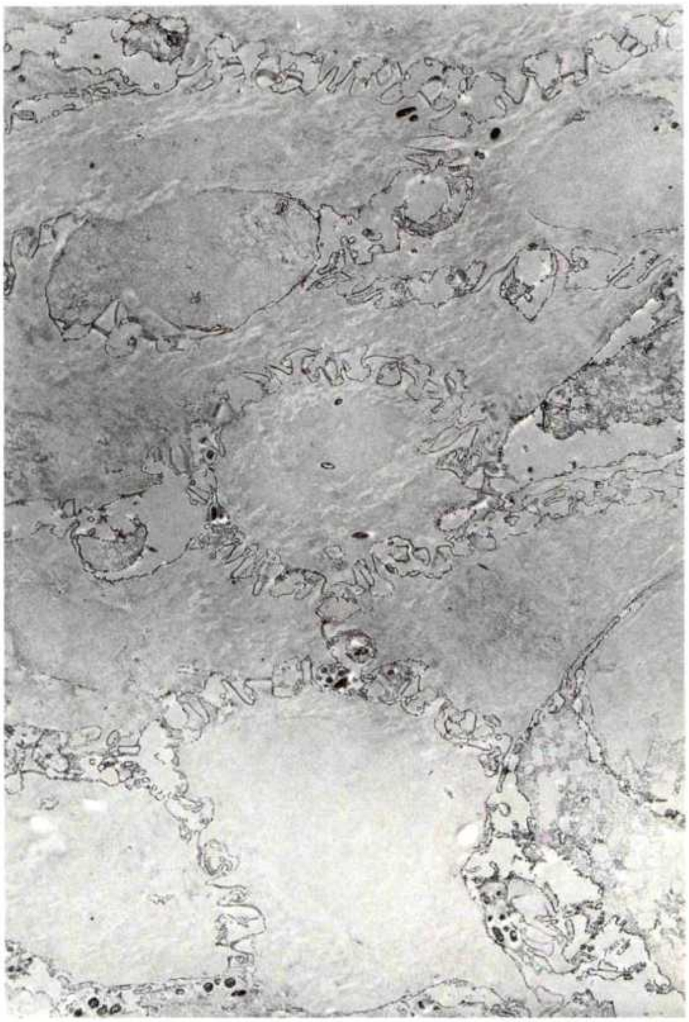

FIGURE 7. Regressing flat warts. By immunoelectron microscopy, using anti-HLA-DR antibody, electron dense deposits were noted on the surface of keratinocytes, dendritic cells and infiltrating cells. (× 6000).

characterized histologically by lymphocytic exocytosis into the epidermis, i.e. pityriasis rosea, spontaneously regressing flat warts, Schamberg's disease, pityriasis lichenoides chronica, and lichen striatus as well as those reported before such as lichen planus and poikilodermatous mycosis fungoides. So we consider that the diffuse intercellular staining of the epidermis with anti-HLA-DR antibody in those six diseases should be an expression of the antigens on the surface of keratinocytes. Furthermore, we confirmed immunoelectron microscopically the actual presence of HLA-DR antigens on the surfaces of keratinocytes in pityriasis rosea and spontaneously regressing flat warts.

Lichen sclerosus et atrophicus (LS et A) showed epidermal intercellular staining with anti-OKT6 antibody rather than with anti-HLA-DR antibody; LS et A does not have any lymphocytic infiltration into the epidermis despite the fact that there is a lichenoid epidermal change. Even though we could not perform immunoelectron microscopy in this case to confirm the exact location of OKT6 antigen, this fact indicates that the immunoperoxidase staining of Langerhans cells with anti-OKT6 antibody also may take an intercellular pattern. Therefore, in order to investigate the expression of HLA-DR antigen on the keratinocyte surface, it is necessary to rule out the involvement of Langerhans cells.

Of the eleven diseases we studied, only pityriasis rosea, regressing flat warts, Schamberg's disease, pityriasis lichenoides chronica, lichen striatus, lichen planus and mycosis fungoides showed the expression of HLA-DR antigen on keratinocyte surface. Compared with the 'negative' diseases such as psoriasis, Jessner's lymphocytic infiltration of the skin, B-cell lymphoma, and lichen sclerosus et atrophicus, the above-mentioned diseases in which keratinocytes express HLA-DR antigens share common histological characteristics such as active exocytosis of lymphocytes and degeneration of keratinocytes (Lever & Schaumberg-Lever, 1983). Although the intercellular distribution of HLA-DR antigens has been reported in focal areas of psoriatic epidermis (Morhenn *et al.*, 1982), we could not find any distinct staining in any of our seven cases; there was weak and ambiguous staining at only a few suprapapillary portions of the epidermis.

We found the presence of a dense infiltration of HLA-DR$^+$ cells under the epidermis that expressed HLA-DR antigens on the keratinocyte surface. In pityriasis rosea, regressing flat warts and lichen striatus, the HLA-DR antigen expression of the keratinocyte surface was confined to the portion of the epidermis where active exocytosis of HLA-DR$^+$ cells took place. Most of these HLA-DR$^+$ infiltrating cells were identified as T lymphocytes by the immunoperoxidase staining technique with a monoclonal anti-T-lymphocyte antibody. The expression of HLA-DR antigen on T lymphocytes is thought to be a marker of activated T lymphocytes (Ko *et al.*, 1979). With regard to regressing flat warts, we have obtained evidence indicating an important role played by cellular immunity (Tagami *et al.*, 1974; Tagami, Oguchi & Ofuji, 1980). Suitters & Lampert (1982) suggested that Ia antigen expression on keratinocytes is closely linked to cellular immunity involving the epidermis. On the basis of this evidence, we suggest that cellular immune reactions may also occur in the lesional epidermis of diseases such as pityriasis rosea, Schamberg's disease, pityriasis lichenoides chronica, and lichen striatus.

There is evidence that keratinocytes may be involved in immune responses, playing a role similar to that of macrophages by secreting an interleukin-1-like factor called epidermal thymocyte activating factor (Luger *et al.*, 1982). Volk-Platzer *et al.* (1983) presented the data favouring the hypothesis that HLA-DR antigens on the keratinocyte surface are actively synthesized by those cells. We think that the expression of HLA-DR antigen on the keratinocyte surface accompanied by exocytosis of active T lymphocytes is a sign of some active role played by those keratinocytes in cellular immunity.

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The effect of short-term corticosteroid incubation on the alloactivating and antigen-presenting capacity of human epidermal Langerhans cells

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SUMMARY

The effects of short-term incubation with hydrocortisone and methylprednisolone on the alloactivating capacity and the ability of human epidermal Langerhans cells and peripheral blood monocytes to induce antigen (PPD)-specific T-lymphocyte responses were investigated. The Langerhans cells and the monocytes were treated with different concentrations of the steroids before being added to allogeneic T lymphocytes, and before, after or simultaneously with, pulsing with PPD. Neither steroid inhibited the alloactivating capacity of the Langerhans cells, but a dose-dependent reduction of the stimulatory capacity of antigen-pulsed Langerhans cells and monocytes was obtained with methylprednisolone. Hydrocortisone had inhibitory effects only at high concentration. The inhibition appeared to be due to suppression of the presentation of PPD in immunogeneic form to sensitized T cells.

Human epidermal Langerhans cells play an important role in the immune responses in the skin. We have previously reported that they can (1) induce allogeneic T-cell responses (Braathen & Thorsby, 1980a); (2) substitute for monocytes in the presentation of antigen in an immunogenic form to sensitized T cells (Braathen & Thorsby, 1980a, 1980b; Braathen *et al.*, 1980; Braathen & Kaaman, 1983); (3) induce strong proliferative T-cell responses to nickel sulphate in sensitized individuals (Braathen, 1980). Furthermore we have shown that Langerhans cells are more potent than blood monocytes in inducing some antigen-specific T-cell responses (Braathen & Thorsby, 1983), and that the antigen responses are restricted by the HLA-D/DR molecules (Braathen & Thorsby, 1982; Berle, Braathen & Thorsby, 1982; Hirschberg, Braathen & Thorsby, 1982).

Glucocorticosteroids possess immunosuppressive effects, and are widely used in dermatology. *In vitro* studies have demonstrated steroid effects on both T cell and monocyte functions (Rosenberg & Lysz, 1978; Haynes & Fauci, 1979; Randazzo *et al.*, 1980; Kraft, Thomson & Atkins, 1979; Barlow & Rosenthal, 1973). They affect the antigen-presenting properties of human monocytes and endothelial cells (Hirschberg *et al.*, 1982) and topical steroids inhibit induction of human contact sensitization (Burrows & Stoughton, 1976). Repeated topical or systemic administration of glucocorticoids in guinea-pigs caused a marked decrease in Langerhans cells, as assessed by staining for cell membrane ATPase activity and Ia antigens (Belsito *et al.*, 1982). Another report showed that epicutaneous steroids reduce the number of Fc-rosetting, C3b-rosetting and Ia-bearing Langerhans cells in a dose-related fashion in guinea-pig, rat, and man (Berman *et al.*, 1983). These alterations of the epidermal Langerhans cells might interfere with the alloactivating and antigen-presenting capacities. We have therefore investigated the *in vitro* effect of short-term incubation with glucocorticosteroids on these functions. Our results indicate inhibition of the antigen-presenting function with high doses of methylprednisolone and hydrocortisone succinate. No effect was seen on the alloactivating ability of Langerhans cells.

METHODS

Cells were obtained from unrelated tuberculin-positive individuals.

PPD solution in phosphate buffer without preservative was obtained from the Veterinary Institute, University of Oslo, and used freshly diluted (2·5 μg/ml) in medium RPMI 1640 supplemented with penicillin and streptomycin.

Methylprednisolone (Solu Medrol®) and hydrocortisone succinate (Solu Cortef®) were obtained from The Upjohn Company, Kalamazoo, Michigan, U.S.A., and used diluted in medium RPMI 1640 with L-glutamine (Gibson-Bio-Cult, Glasgow).

Epidermal cells

Epidermal cell suspensions were made by enzymatic digestion of epidermis sheets obtained with the suction blister technique as previously described (Braathen & Thorsby, 1980a). The cells were suspended in medium RPMI 1640 with L-glutamine (Gibco-Bio-Cult, Glasgow) supplemented with penicillin, streptomycin and 20% normal human serum. Viability, determined by trypan blue exclusion, ranged from 75% to 95%, but was usually more than 90%. The cell suspensions contained 2–6% Langerhans cells, as judged by immunofluorescence staining of the cell suspensions with a rabbit anti-DR antiserum as described earlier (Braathen & Thorsby, 1980a).

T lymphocytes

Peripheral blood mononuclear cells were separated from defibrinated blood by means of flotation on Lymphoprep® (Nyegaard & Co., Oslo, Norway). T lymphocytes were prepared from the isolated mononuclear cells by rosetting with AET-treated sheep red cells as described earlier (Braathen & Thorsby, 1980a).

Monocytes (Mo)

Monocytes were prepared from isolated mononuclear cells by means of an adherence technique as previously described (Braathen & Thorsby, 1980a).

Incubation of epidermal cells with steroids and PPD

Epidermal cells (5×10^4) were either (i) first incubated with various concentrations of the steroids for 90 min at 37°C, then washed twice before being incubated another 90 min at 37°C with PPD and after 3 washes resuspended in medium RPMI 1640 with 20% normal human serum, or (ii) the epidermal cells were first incubated with antigen and then with steroids, or (iii) incubated for 15 min at 37°C with steroids before antigen was added and left with both the steroid and antigen for 90 min at 37°C before being washed 3 times and resuspended in medium RPMI 1640. Various numbers of epidermal cells were also incubated with steroids for 90 min at 37°C, before being used in cultures or stained for DR and T6 antigens. Control epidermal cells went through the same procedures using steroid-free medium RPMI 1640. Some of the cultures had antigen present during the entire incubation. The incubation experiments were performed both in tubes and in the wells of microtitre plates.

Staining of epidermal cell suspensions (Ec) with anti-DR and OKT6 antisera

Ec were stained with anti-DR antiserum and a fluorescein isothiocyanate (FITC)-conjugated swine anti-rabbit antiserum as previously described (Solheim *et al.*, 1978). Staining for T6 antigen was performed by first incubating the Ec with OKT6 at 4°C for 30 min and then, after two washings, incubating them for another 30 min at 4°C with a FITC-conjugated sheep anti-mouse antiserum. The cells were stained after incubation for 90 min at 37°C with the various concentrations of methylprednisolone and hydrocortisone. As controls we used unincubated cells and cells that were incubated with medium alone without steroids. More than 200 cells were counted in each preparation.

Cell culture techniques

The cells were incubated in round-bottomed microtitre plates (Model 15-MCR 96, Linbro Scientific Inc., Hamden, Conn., U.S.A.) in a humid 5% CO_2 atmosphere at 37°C for 5 days. The epidermal cells treated with steroids alone were co-cultured with 5×10^4 allogeneic T cells for 5 days. The epidermal cells that were PPD-pulsed before, after or simultaneously with steroid treatment were co-cultured with 5×10^4 autologous T cells for 5 days. Each well contained 170 μl medium RPMI 1640 supplemented with antibiotics and 20% normal human serum. [^{3}H]thymidine was added 18 h before harvesting with a semi-automatic multiple cell culture harvester (Skatron, Lierbyen, Norway). Incorporation was measured as median c.p.m. of triplicate cultures.

RESULTS

Effect of preincubation of Langerhans cells with steroids on the alloactivating activity

In Table 1 the results of experiments using steroid- and non-treated epidermal cells to induce allogeneic T-cell responses are shown. The table shows the results obtained with methylpredni-

TABLE 1. No significant effect of preincubation of epidermal cells with methylprednisolone (Mp) for 90 min at 37°C on alloactivation of T cells

T cells alone	No. of epidermal cells (× 10^4)	Concentration of Mp (μg/ml)					
		0	0·01	0·1	1·0	10·0	100·0
533[a]	2	4754	6104	5414	7040	5714	5064
533	5	8228	7708	7884	6762	7552	6660

[a] Median c.p.m. of triplicate cultures.

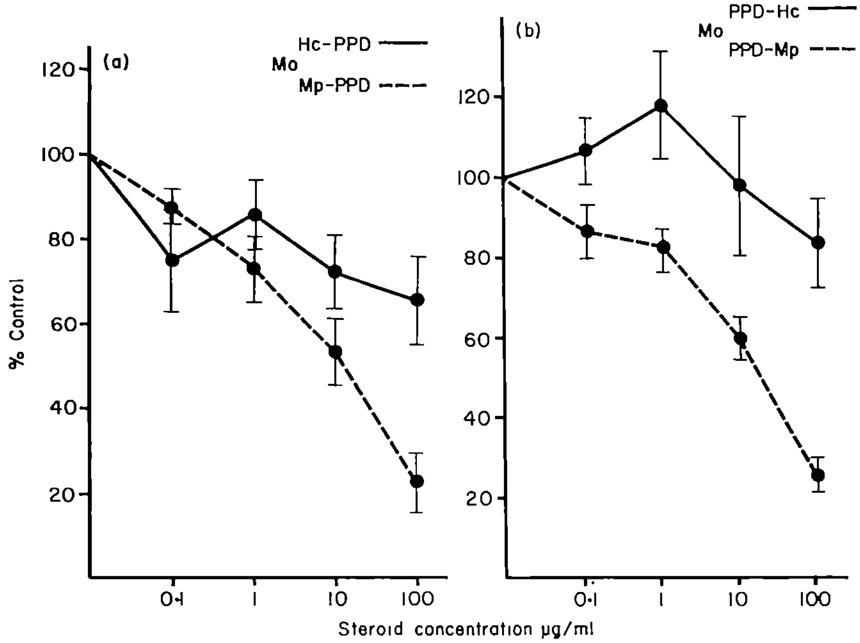

FIGURE 1. Effects of steroids on monocytes (Mo). 10^4 monocytes were either first incubated with various concentrations of Hc (●——●) or Mp (●– – –●) before pulsing with PPD (a) or were pulsed with PPD and then treated with Hc or Mp (b). The results are presented as percentage of control cultures using non-steroid-treated monocytes. Average of three experiments ± s.e.

solone (Mp). Similar results were obtained with hydrocortisone (Hc). No difference was found between the alloactivating capacity of steroid-treated and untreated Langerhans cells.

Steroid inhibition of the Langerhans cell-dependent and monocyte-dependent induction of T-cell responses to PPD

Figure 1 shows the effect of steroids on the antigen-presenting capacity of monocytes. In Figure 1a the results of experiments are shown in which the monocytes were first incubated with steroids and then with antigen. Figure 1b shows the results of experiments in which the monocytes were incubated with antigen before the steroid. Significant and dose-dependent inhibitions of the induction of PPD specific T-cell responses were observed for the highest concentrations of Hc and concentrations of Mp over 1 µg/ml.

Figure 2 shows the effect of steroids on the antigen-presenting capacity of Langerhans cells. In Figure 2a the results of experiments are shown, in which the Langerhans cells were first treated with steroids and then incubated with PPD, and Figure 2b shows results from steroid treatment after antigen pulsing. The results of experiments with simultaneous antigen pulsing and steroid treatment were similar (data not shown). Significant and dose-dependent inhibitions were obtained with Mp and the highest concentrations of Hc, and no differences were observed whether the cells were incubated with steroids before, after or simultaneously with antigen-pulsing.

Viability of the treated Langerhans cells and monocytes

To exclude the possibility that the inhibition of the Langerhans cell and monocyte function

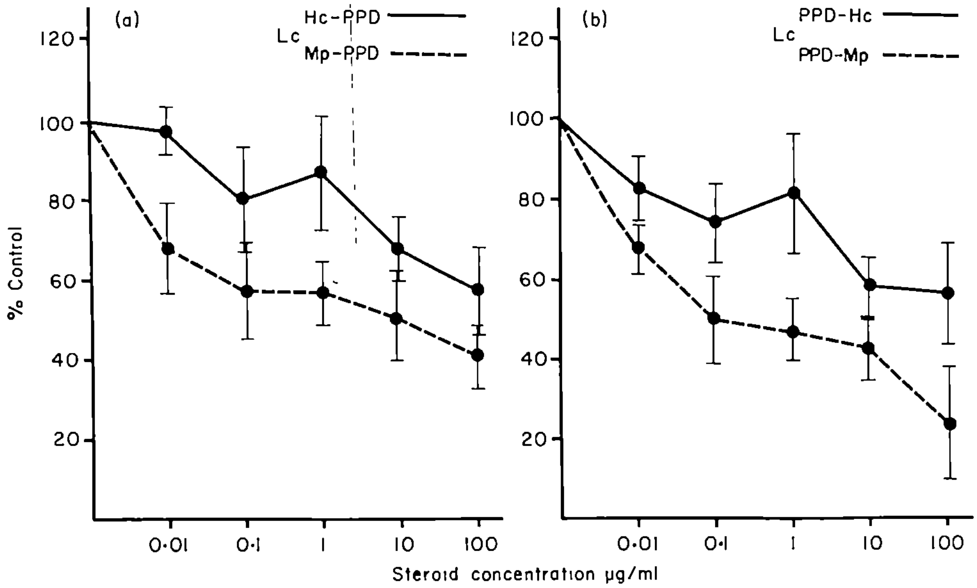

FIGURE 2. Effects of steroids on Langerhans cells (Lc). 5 × 10^4 epidermal cells were first incubated with various concentrations of Hc (●——●) or Mp (●– – –●) before pulsing with PPD (a) or were pulsed with PPD and then treated with Hc or Mp (b). The results are presented as percentage of control cultures using non-steroid-treated Langerhans cells. Average of five experiments ± s.e.

observed was due to a direct toxic effect of the steroids on the cells, we performed experiments in which steroid-treated and antigen-pulsed Langerhans cells and Mo were incubated with T cells in the continuous presence of antigen in the wells for the entire culture period. The results from a typical experiment with Langerhans cells are presented in Table 2. It can be seen that the steroid inhibition can be completely reversed when antigen was present in the medium during the culture period. This was the case also for the highest concentration (100 µg/ml) of Hc and Mp that was tested, indicating that the steroids had no toxic effect on the Langerhans cells and furthermore, that 90 min incubation with steroids did not irreversibly inhibit the antigen-presenting function. Similar results were obtained with Mo (data not shown).

Counts of DR and OKT6-positive cells in the epidermal cell suspensions after steroid incubation

Immunfluorescence staining of the epidermal cell suspensions demonstrated the same number of DR and OKT6-positive cells in the unincubated cell suspensions as in the cells that were incubated with medium without steroids, as well as in the cell suspensions that were incubated with the various concentrations of steroids.

DISCUSSION

The present study demonstrates an effect of glucocorticosteroid hormones on the ability of human epidermal Langerhans cells and monocytes to induce antigen-specific T-cell responses.

One explanation for our results could be a direct toxic effect of steroids on the Langerhans cells and monocytes. We therefore performed experiments in which antigen was continuously present in the cultures. The data from these experiments (Table 2) indicate that there were no toxic effects of the steroids on the cells, since the steroid-induced inhibition could be reversed;

TABLE 2. Viability of steroid-treated Langerhans cells. PPD was added to the wells after the cells had been either steroid-treated and then PPD-pulsed, or PPD-pulsed and then steroid-treated, or simultaneously PPD-pulsed and steroid-treated. Hc = hydrocortisone; Mp = methylprednisolone

Ec plus PPD added	PPD-pulsed Ec	Conc. of Hc or Mp (μg/ml)	Steroid-treated and then PPD-pulsed[a]		PPD-pulsed and then steroid-treated		Simultaneously PPD-pulsed and steroid-treated	
			−PPD[b]	+PPD[b]	−PPD	+PPD	−PPD	+PPD
10,881	7583	Hc 1	6003	12,571	6849	8954	6286	10,702
10,881	7583	Hc 10	5266	13,776	5157	12,322	6144	8980
10,881	7583	Hc 100	5405	13,236	4298	7428	4535	9801
10,881	7583	Mp 1	6748	12,570	5269	12,510	6157	11,200
10,881	7583	Mp 10	6635	13,077	3245	12,277	5984	13,236
10,881	7583	Mp 100	4328	10,023	2574	13,879	5168	10,682

[a] T-cell responses are expressed as median c.p.m of triplicate cultures. T cells alone: 345. T cells plus PPD: 826.

[b] Without PPD in the cultures (−PPD); with PPD in the cultures (+PPD).

and furthermore, it indicates that the effects of the steroids are reversible. Our results also show that the inhibitory effects of the steroids were similar whether the cells were incubated with steroids before, after or simultaneously with antigen.

No effect was seen on the alloactivating activity using the experimental conditions described here, but we cannot exclude the possibility that the steroids had a time-limited effect on the alloactivating activity, and that the full alloactivating activity was restored after the removal of steroids. The results are similar to those obtained with PPD continuously present in the cultures with steroid-pretreated Ec and T cells. No significant difference was obtained between the T-cell responses obtained with untreated or steroid-treated Ec (Table 2).

The induction of antigen-specific T-cell responses by Langerhans cells is complex and probably involves uptake of antigen, some form of processing or degradation, and eventual antigen presentation in the cell membrane. The class II HLA-DR molecules are important for antigen activation. The activation of T cells involves co-recognition of antigen and HLA-D region products in the membrane of antigen-presenting cells (Berghotz & Thorsby, 1977, 1978; Thorsby, Berle & Braathen, 1983). We have previously shown that HLA-D/DR antigens restrict the Langerhans cells dependent antigen activation of T cells (Braathen & Thorsby, 1982; Berle *et al.*, 1982; Hirschberg, Braathen & Thorsby, 1982), and that blocking of the DR antigens on the surface of the Langerhans cells with anti-DR antiserum abolishes the ability to induce T-cell responses (Braathen, 1980).

Recent reports have demonstrated a decrease in the expression of Langerhans cell Ia antigens after repeated topical or systemic administration of glucocorticoids (Belsito *et al.*, 1982; Berman *et al.*, 1983). To investigate whether the steroid incubation we performed had decreased or abolished the membrane HLA-D/DR or T6 molecules of the Langerhans cells, we stained the epidermal cell suspensions for DR and T6 antigens before and immediately after incubation with the steroids. No differences were observed in the counts of DR- and T6-positive cells before and after incubation. However, even if the number of DR-positive cells remained the same, we cannot exclude the possibility of a reduction of DR molecule density in the cell membrane, which possibly could explain the observed inhibitory effect. The previously reported decrease in Langerhans cell density first appeared after 2 days of steroid treatment and furthermore seemed to be an 'all-or-nothing' phenomenon (Belsito *et al.*, 1982).

Inhibition was observed with steroid treatment after the monocytes and Langerhans cells had sufficient time to take up the PPD, which might indicate that the steroids inhibit either the presentation of antigen on the surface of the cells or inhibit the production of soluble factors necessary for T-cell proliferation.

Presence of PPD in the medium during the entire culture period could reverse the steroid-induced inhibition, indicating that the inhibition may be effective only within a certain period of time during and after the steroid treatment. After that time the cells were able to take up, process and then present the PPD to T cells.

The doses of steroids used in this study are well above pharmacological levels, and indicate that the antigen-presenting function of Langerhans cells and monocytes is relatively steroid resistant. Topical treatment with highly potent glucocorticosteroids is frequent in skin diseases. Only a small part of the total amount of topically applied steroids goes into the skin; but repeated applications over some time seems to reduce the expression of D/DR antigens (Belsito *et al.*, 1982; Berman *et al.*, 1983) and might therefore affect the antigen presentation of the Langerhans cells.

The profound clinical effect of potent topically applied and systemic steroids on inflammatory skin diseases must, however, be explained by an influence of other mechanisms as well.

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Establishment of T and B cell lines from patients with mycosis fungoides

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SUMMARY

Eighteen patients with mycosis fungoides were studied in order to establish cell lines that might be associated with the human T cell leukaemia-lymphoma virus (HTLV). Three T cell lines were established, two from affected skin and one from a lymph node showing dermatopathic lymphadenopathy. The T cells expressed OKT3 and OKT4 antigens. They temporarily expressed an HTLV p19-like antigen in up to 5% of the cells during culture. None of our patients had lymphocytosis or abnormal lymphocytes, except one patient with Sézary's syndrome. We could not establish T cell lines from peripheral blood, but five B lymphoblastoid cell lines were obtained, all positive for the Epstein–Barr virus nuclear antigen.

Our finding that T cell lines can be established from skin biopsies and lymph nodes of patients with mycosis fungoides, but not from the blood, supports the concept of a malignant T lymphocyte primarily localized in the skin. The temporary expression of HTLV p19 antigen may indicate the presence of retrovirus, but further studies are needed.

In 1979 van der Loo and co-workers observed retrovirus particles in Langerhans cells in skin biopsies from patients with mycosis fungoides and Sézary's syndrome. Poiesz *et al.* (1980) isolated and characterized a retrovirus (HTLV) from the T lymphocyte cell line of a patient with cutaneous T cell lymphoma. Within the last 3 years HTLV has been isolated from patients with cutaneous T cell lymphoma and adult T cell leukaemia (Popovic *et al.*, 1983; Miyoshi *et al.*, 1981; Bunn *et al.*, 1983).

These studies have prompted us to look for HTLV in Scandinavian patients with mycosis fungoides (MF). As a first step towards this aim, we have established T cell lines from skin and lymph nodes and B cell lines from peripheral blood and lymph nodes from patients with MF.

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METHODS

Eighteen patients were studied, eight women and ten men, in the age range 42 to 85 years. A diagnosis of MF was made only when histological examination of affected skin showed Pautrier's microabscesses in the epidermis and a band-like pleomorphic, mononuclear dermal infiltration containing some cells with indented nuclei. All biopsy specimens were reviewed by one pathologist (Dr Helmer Søgaard). The clinical stage classification was that of van Scott and Kalmanson (1973). Stage II is erythematous, scaling plaques in the skin without skin tumours or lymph node involvement; stage IIIb is tumour formation in the skin and dermatopathic lymphadenopathy; and stage IV is patients with plaques and tumours in the skin and malignant lymphoma in their lymph nodes. None of the patients studied had lymphocytosis or Sézary cells in peripheral blood, except the patient with Sézary's syndrome (mycosis fungoides 18, Table 1).

The patients' data are shown in Table 1. Most of the patients had received therapy before they were studied. Skin and lymph node biopsies were taken from patients with newly developed skin lesions or lymph node enlargement before therapy was given.

Blood lymphocytes from 20 ml of heparinized blood were isolated by the standard Ficoll–Isopaque separation technique and resuspended in RPMI 1640 with 10% fetal calf serum (FCS), 10% human serum and 25% crude T-cell growth factor. Briefly, the T-cell growth factor was prepared by seeding isolated human lymphocytes at $2{\cdot}5 \times 10^6$/ml in RPMI 1640 with 10% FCS and adding 5 μg/ml of phytohaemagglutinin (PHA-P) (Difco) and 10 ng/ml of phorbol-12-myristate-13-acetate (TPA). Cells were incubated for 3 hours, then extensively washed and incubated in RPMI 1640 with 10% FCS for 40 hours. The supernatant was harvested and used as a source of crude T-cell growth factor.

The skin biopsies were taken under local anaesthesia with lidocaine. Each biopsy was approximately 5 × 10 mm. The skin tissue and lymph node biopsies were cut into small pieces, but were not subjected to further chemical or physical homogenization. The material was placed in 5 ml medium (described above) and incubated at 37°C in a 5% CO_2 atmosphere. The medium was replaced twice weekly. If cell density was below 2×10^5/ml, cells were recovered by centrifugation and resuspended in 5 ml fresh T-cell growth factor-containing medium. When cell density ranged between 2×10^5/ml and 5×10^5/ml, cultures were split in a ratio of 1:2. All cultures were free of mycoplasma detected by the method of Chen (1977). When cell growth was observed beyond 3 months, the culture was considered a cell line.

In some experiments OKT8-positive lymphocytes were eliminated before the start of cell culture using an anti-OKT8 monoclonal antibody and rabbit complement (Reinherz *et al.*, 1980). Staining for surface markers (OKT3, OKT4, OKT8) was done as described by the supplier (Ortho Pharmaceuticals), as was staining for surface immunoglobulin (DAKO Immunoglobulins). Epstein–Barr virus nuclear antigen (EBNA) staining was done according to the method of Reedman and Klein (1973). Indirect immunofluorescence staining for HTLV p19 was performed using a monoclonal antibody kindly supplied by Drs Marjorie Robert-Guroff and R.C.Gallo, as described by Robert-Guroff *et al.* (1981).

RESULTS

Twenty-three blood cultures were examined. When the blood lymphocytes were grown as described, proliferation of cells generally ceased after 6 weeks in culture. In five cases cell lines were established. The cells had surface immunoglobulin (IgM) and stained positive for EBNA.

TABLE 1. Patients with mycosis fungoides (MF) where attempts were made to establish continuously growing cell lines

Patient	Sex	Age (years)	Stage of disease	Duration of disease (years)	Disease activity	Previous therapy	Date of sample	Source	Cell line established	Course of disease
MF 6	F	88	II	9	Active	Mustine	27/5/81	Blood	—	Died 10/8/81, no MF
MF 7	M	74	II	16	S. active	Mustine	30/7/81	Blood	—	
MF 8	M	79	II	16	Inactive	None	31/7/81	Blood	—	
MF 9	F	85	II	20	S. active	PUVA	14/8/81	Blood	—	
MF 10	F	73	II	14	Inactive	Mustine	21/8/81	Blood	—	
MF 11	F	62	II	20	Inactive	PUVA	25/8/81	Blood	B cell line	
MF 19	M	84	II	4	Active	None	15/1/82	Blood	—	
							15/1/82	Lymph node	—	
MF 20	F	56	II	10	S. active	Mustine	15/1/82	Blood	—	
MF 21	M	86	II	12	Active	Mustine		Blood	—	
MF 22	M	72	IIIb	8	Active	None	25/8/82	Skin	T cell line	
MF 4	F	63	IIIb	7	V. active	None	8/12/80	Blood	—	
					V. active	BCP-R+e.b.	26/6/81	Blood	—	
					V. active	BCP-R+e.b.	24/7/81	Blood	B cell line	
					V. active	BCP-R+e.b.	22/8/81	Blood	B cell line	
MF 13	F	43	IIIb	13	Active	None	2/9/81	Blood	B cell line	
					Active	None	21/6/83	Lymph node	T cell line	
MF 1	M	45	IV	3	Inactive	None	27/5/81	Blood	—	
					Active	Mustine	5/9/81	Blood	—	
					Active	None		Skin	T cell line	
MF 2	M	65	IV	5	Active	None	27/5/81	Blood	—	Died 7/9/82 of MF
					V. active	Mustine	27/6/81	Blood	—	
MF 3	M	42	IV	12	Active	None	27/6/81	Blood	—	
					V. active	Mustine	5/1/82	Blood	—	
					V. active	Mustine	5/1/82	Lymph node	—	
MF 5	F	62	IV	8	Inactive	Mustine	30/6/81	Blood	—	
MF 12	M	59	IV	9	Active	Mustine	31/8/81	Blood	—	Died 28/12/82 of MF
MF 18	M	70	SS	10	Active	Pheresis	18/12/81	Blood	B cell line	

S = slightly.
V = very.

They were therefore B lymphoblastoid cell lines. From none of the patients could we establish T cell lines from peripheral blood.

In contrast to other B lymphoblastoid cell lines, we observed difficulties with continuous growth. This occurred simultaneously with the development of chromosomal end-to-end translocations. These changes occurred in all five cell lines and will be described in detail elsewhere.

We obtained skin biopsies from two patients with newly developed nodules in their skin, one in stage IIIb and one in stage IV. During the first 3 weeks of culture, lymphocytes could be seen migrating out from the pieces of skin tissue. At this time approximately 80% of the cells had the OKT4 and 20% the OKT8 phenotype. All cells were positive for OKT3. This indicates that no major selection of T lymphocyte subpopulations had occurred, in agreement with the results of Willemze *et al.* (1983).

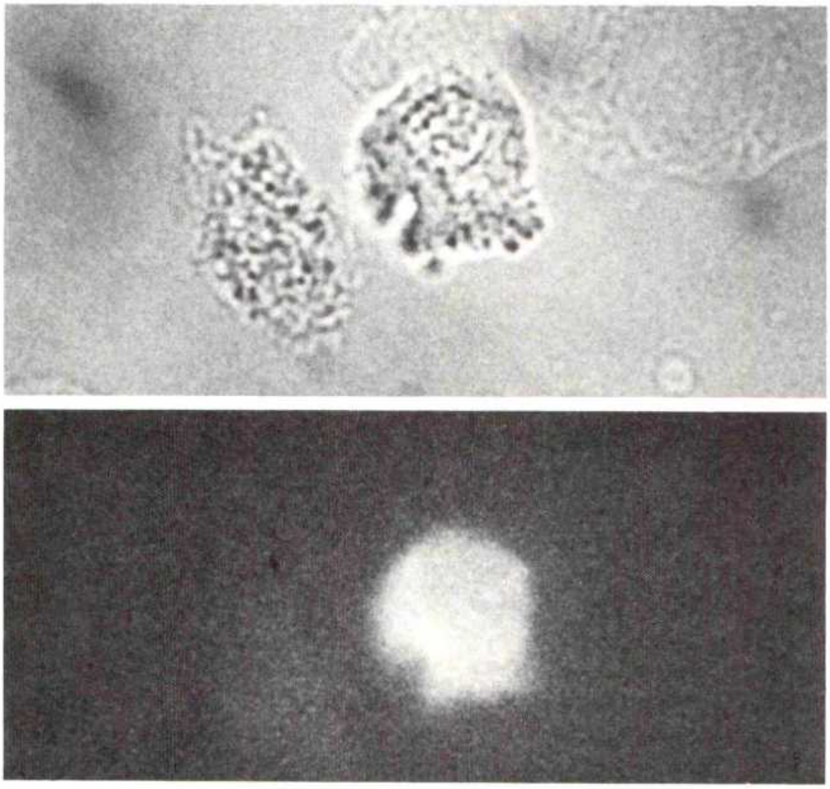

FIGURE 1. Indirect immunofluorescence assay. Lymphocytes obtained from the skin biopsy of patient MF 1 and cultured for 14 days were washed with PBS, cytocentrifuged, air-dried and fixed for 10 min at room temperature in 50% methanol:50% acetone. Control ascites of P3 × 63 cells and ascites of hybridoma 12/1-2 produced as described by Robert-Guroff *et al.* (1981) were diluted 1:400 in PBS and applied to the cells for 30 min. After washing the cells three times with PBS, fluorescein-conjugated rabbit immunoglobulins to mouse immunoglobulins (Dako, Denmark) diluted 1:10 in PBS with 5% pooled human serum was applied to the cells for 30 min. After washing the cells three times with PBS the cells were examined microscopically. Cells treated with P3 × 63 diluted serum showed no fluorescence, whereas 2% of the cells stained with the diluted 12/1-2 serum showed a bright fluorescence. The figure shows two cells as seen in light microscopy (upper) and by fluorescence microscopy (lower). Only one of the two cells fluoresced (× 1250).

Up to 5% of the cells expressed a clearly positive cytoplasmic staining using HTLV p19 antibody (Fig. 1). By further cultivation the HTLV p19 antigen disappeared concomitant with the disappearance of cells expressing the OKT4 phenotype. This gave rise to a cell culture of OKT8-positive cells with a limited life span of less than 3 months. Initial elimination of OKT8-positive cells gave rise to an OKT4-positive cell line. The cells expressed HTLV p19 antigen in a low percentage, but the antigen disappeared after approximately 6 weeks of culture.

The cell line from a lymph node showing dermatopathic lymphadenopathy had more than 70% of OKT4-positive lymphocytes. Following our experience from the skin cultures, lymphocyte cell suspensions were treated with anti-OKT8 plus complement to remove the OKT8-positive cells. This leads to outgrowth of OKT4-positive lymphocytes. Again, we found a temporary expression of HTLV p19 antigen as seen in the skin lymphocyte cultures. If the OKT8-positive lymphocytes were left in culture, no cell line could be established.

The cell lines from skin and lymph node are mature T cells as judged by their positive reaction with OKT3 and OKT4 antibody and E-rosetting capacity. They lack surface immunoglobulin and Epstein–Barr virus nuclear antigen.

DISCUSSION

Mycosis fungoides (MF) presents with red, circumscribed scaling plaques often accompanied by severe pruritus. Initially, it is limited to the skin. It has a relapsing course, which may extend over decades, but ultimately it progresses into a malignant lymphoma with tumour formation in the skin, and involvement of regional lymph nodes and internal organs (Vonderheid, 1980; Green, Byar & Lamberg, 1981).

The studies by Gallo's group have strongly suggested that MF is caused by retrovirus. They have described a retrovirus in T lymphocyte cell lines from lymph nodes and blood (Poiesz *et al.*, 1980; Popovic *et al.*, 1983). Their patients had cutaneous T cell lymphoma, which corresponds to the patients in the malignant stage of MF (stage IV).

In agreement with these observations we have been able to establish T cell lines with OKT4 phenotype from lymph nodes, but only after removal of OKT8 lymphocytes before the start of cell culture. Preliminary evidence suggests that the OKT8-positive cells are cytotoxic T cells specific for an antigen present on epithelial cells, fibroblasts and the OKT4-positive cells in the culture. In contrast, it was not possible for us to obtain T cell lines from peripheral blood (considered to be a non-malignant tissue), even from patients with MF stage IV from which OKT8-positive cells were present or removed. We must, however, stress that there was no lymphocytosis and no evidence of malignant cells in the blood from our patients.

It is a new observation that T cell lines can be established from skin biopsies in MF. We believe that these OKT4-positive cell lines are important to study. MF starts in the skin and is considered to be a cutaneous lymphoma of T helper cells (Worman, Burns & Barker, 1978; Edelson, 1980). Our establishment of T cell lines from skin, but not from blood, supports the concept that the malignant lymphocytes develop in the skin, and not in the blood.

The detection of HTLV p19-like antigen in primary, fast-growing lymphocyte cultures from skin and lymph nodes may indicate a retrovirus involvement. The lack of antigen expression, when a cell line is established, may have several explanations. Antigen expression in slow-growing cells may be beyond the detection level by the indirect immunofluorescence method, if the antigen is not HTLV p19 but a cross-reactive antigen. Clonal selection may occur, and the cells may no longer possess the ability to express the HTLV p19-like antigen. Finally, the HTLV p19 antibody may detect a maturation antigen of T lymphocytes, because it reacts with a thymic epithelial antigen (Hayes *et al.*, 1983). Further studies are needed to clarify these points.

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Sweat production in the isolated eccrine sweat gland of the rat

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SUMMARY

A method has been developed to isolate a single rat eccrine sweat gland, together with the attached sweat duct, and to induce sweat secretion directly from the gland *in vitro*. Sweat production was induced using carbachol (2×10^{-5} M to 3×10^{-5} M) and methacholine (2×10^{-5} M) in isolated plantar sweat glands maintained in a suitable environment (modified Krebs–Ringer bicarbonate at 38°C containing 30% w/v fresh rat serum). The output of sweat was measured every 5 minutes for 85 minutes and the maximal final volume obtained with any one gland was 23 nl. Sweat production ceased on administration of appropriate concentrations of atropine (1×10^{-5} M to 5×10^{-5} M), withdrawal of calcium ions from the bathing fluid, and reduction of the medium temperature to 4°C.

There have been several attempts to introduce new methods into the field of eccrine sweat gland physiology and pharmacology. For example, Schulz (1969) introduced the micropuncture method, Sato (1973) studied the microinduction of sweat secretion from an isolated eccrine sweat gland *in vitro* and Mangos (1973) microperfused the sweat duct *in vitro*. It was decided to choose one of these methods, modify it and then apply it to the study of sweat secretion in the rat eccrine sweat gland.

METHODS

Male Wistar rats weighing between 200 and 300 g were killed and their foot pads were excised and rinsed in several changes of ice-cold Ringer solution which contained the following: 145 mM NaCl, 5·0 mM KCl, 1·2 mM $MgSO_4$, 1·0 mM $CaCl_2$, 25 mM $NaHCO_3$, 1·2 mM NaH_2PO_4, 5·5 mM glucose. The solution was gassed with 95% O_2 and 5% CO_2. In addition, 30% v/v freshly prepared rat serum which had been heated to 56°C for 30 minutes and allowed to cool before use was added to the modified Krebs–Ringer solution. Each piece of skin was examined in a bath of Ringer solution at 4°C under a dissection microscope (Nikon, Japan) at × 80 magnification for a

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suitable gland for excision. Unstained glands were dissected using sharp steel needles and Dumont No. 5 forceps and were then cleared of any adhering tissue. Glass pipettes with a fine constriction mounted on the end of a 100 μl Hamilton glass syringe were placed in a micromanipulator (Narishige, Japan) and the duct of the excised eccrine sweat gland was carefully drawn up into the end of the pipette. The pipettes were pulled using an automatic C.F.P. micropipette puller, and the glass capillaries used were obtained from Jencons, Hemel Hempstead, U.K. (2 mm outer diameter and 1–1·25 mm bore).

The syringe was mounted on a rigid metal holder in a specially constructed organ bath. Some liquid paraffin containing 1% w/v Scarlet Red (Searle, U.K.) was injected above the Ringer solution in the pipette (Fig. 1). The gland was then left at 4°C for 10 minutes in order to establish that no leaks were present; if no leakage occurred during this time, the temperature was then raised to 38 ± 0·5°C. It was hoped that the mineral oil would retard Ringer solution and sweat evaporation, and since it was injected up to the Ringer level in the inner bath little hydrostatic pressure difference occurred between the outside and the inside of the pipette.

The sweat gland was then incubated at 38°C for 15 minutes during which time readings were taken using the microscope eyepiece graticule every 5 minutes to detect any further leakage of the medium. If none occurred, 0·1 ml of carbachol hydrochloride (Aldrich, U.S.A.) or methacholine bromide (Sigma, U.S.A.) was injected into the organ bath giving a final concentration of 10^{-6} to 10^{-5} M. The gland was then observed and readings were taken of any upward movement of the solution in the micropipette every 5 minutes for the next hour.

In some experiments, atropine sulphate 1×10^{-5} M to 5×10^{-5} M (Sigma, U.S.A.) was injected in a dose volume of 0·1 ml, 30 minutes following carbachol or methacholine administration, and then readings were continued for the following 30 minutes. In some cases, atropine administration was replaced by changing the Ringer solution to one which contained the same concentration of methacholine or carbachol but no calcium ions. Following a

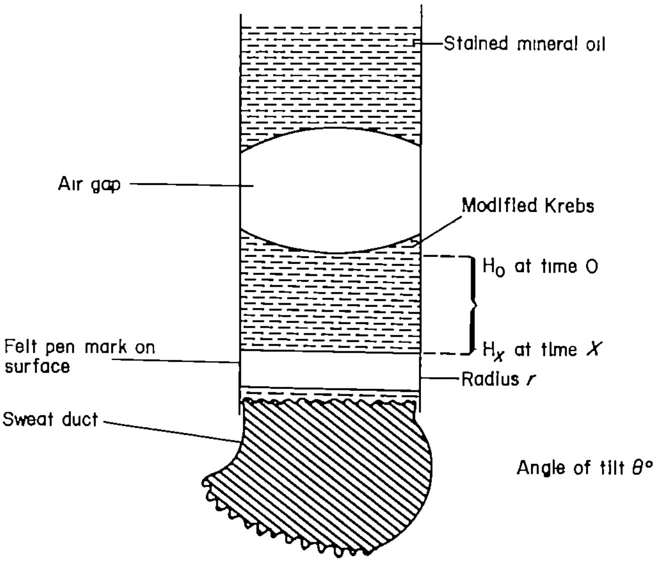

FIGURE 1. Schematic illustration of the method for measuring the volume of sweat output from a single excised rat gland.

15-minute equilibration period readings were taken of any change in the level of the meniscus in the pipette for the next 30 minutes. In some experiments the Ringer solution was changed to one with the usual concentration of calcium ions but was at a temperature of 4°C. Again measurements were made for the ensuing 30 minutes.

All readings (Fig. 1) were initially adjusted for the angle difference of the micropipette (constant angle θ of 30°). The diameter of the pipette was measured at the level of the Ringer solution meniscus; hence $(\text{radius})^2$ was calculated. The distance of the Ringer meniscus from a felt pen mark on the surface of the pipette was measured as $(H_x - H_0)$ at time zero and time x following sweat induction. The volume (V) of sweat secreted V (in nl) at time x was equal to the volume of a cylinder with radius r and height $(H_x - H_0)$:

$$V = \frac{\pi r^2 (H_x - H_0)}{1000}.$$

In order to check the accuracy of the method the results obtained were verified using 1 µl and 10 µl precision Hamilton glass syringes.

RESULTS

Both carbachol (2×10^{-5} M to 3×10^{-5} M) and methacholine (2×10^{-5} M) were found to induce sweating in the rat excised sweat glands maintained in modified Krebs–Ringer solution at 38°C (Figs 2, 3 and 4). The glands produced no secretion at 38°C for the initial 15-minute control

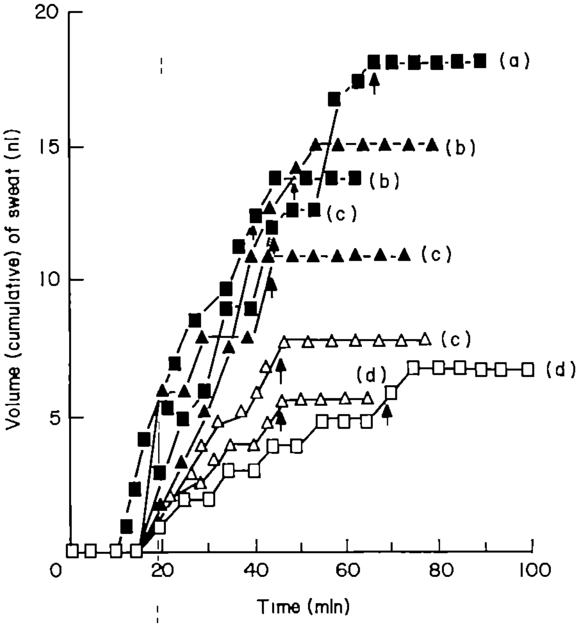

FIGURE 2. Effect of atropine on carbachol-induced sweat secretion in eight isolated sweat glands of rat. Arrows indicate times of atropine addition to the bathing medium, carbachol having been administered at 15 minutes. Concentrations of carbachol (C) and atropine (A) are as follows:
(a) C, 2×10^{-5} M; A, 2×10^{-5} M,
(b) C, 3×10^{-5} M; A, 3×10^{-5} M,
(c) C, 3×10^{-5} M; A, 2×10^{-5} M and
(d) C, 2×10^{-5} M; A, 2×10^{-5} M.

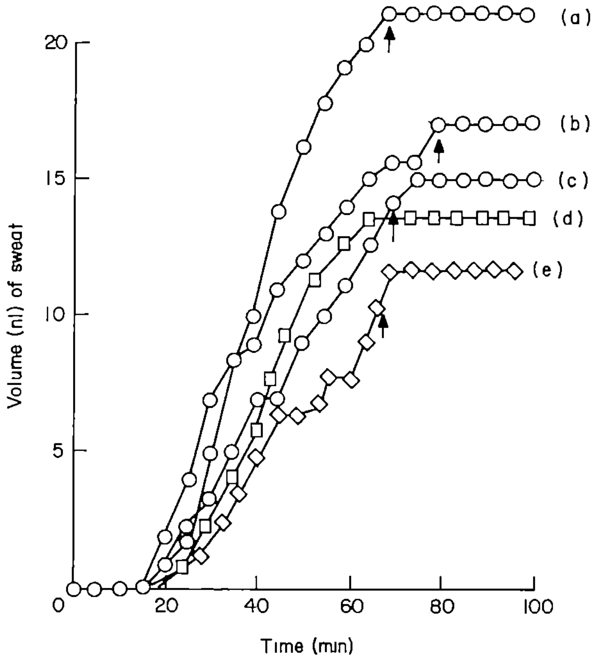

FIGURE 3. Responsiveness of five isolated rat sweat glands to atropine (4 × 10^{-5} M) following stimulation with methacholine (2 × 10^{-5} M) added to the incubation medium at 15 minutes. Arrows indicate times of atropine addition.

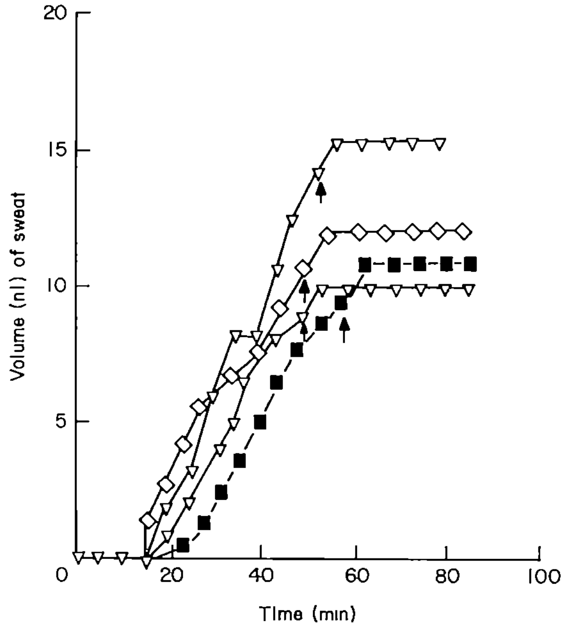

FIGURE 4. Effect of calcium ion lack on methacholine-induced sweat secretion *in vitro*. At times indicated by arrows the incubation medium was replaced by one lacking calcium ions. At 15 minutes methacholine (2 × 10^{-5} M) was added to the bathing fluid.

period prior to drug administration. However, some glands produced 2–9 nl of sweat during the time of temperature adjustment from 4°C to 38°C. Expansion volumes of 1–2 nl following the same temperature rise were observed in identical control micropipettes heat-sealed at one end and containing Ringer solution. Atropine (2×10^{-5} M 4×10^{-5} M) and low medium temperature (4°C) were found to inhibit carbachol and methacholine-induced sweat production (Figs 2, 3 and 5). The sweat glands were found to respond more readily to stimulation of sweating following the administration of methacholine than that following a carbachol dose. Cessation of sweat production was observed when the Ringer solution was replaced by one at 4°C or one containing no calcium ions (Figs 4 and 5).

It is suggested that this modification of the method introduced by Sato (1977) for isolated sweat glands is more satisfactory, but considerable development is needed in order to improve sweat gland response and viability.

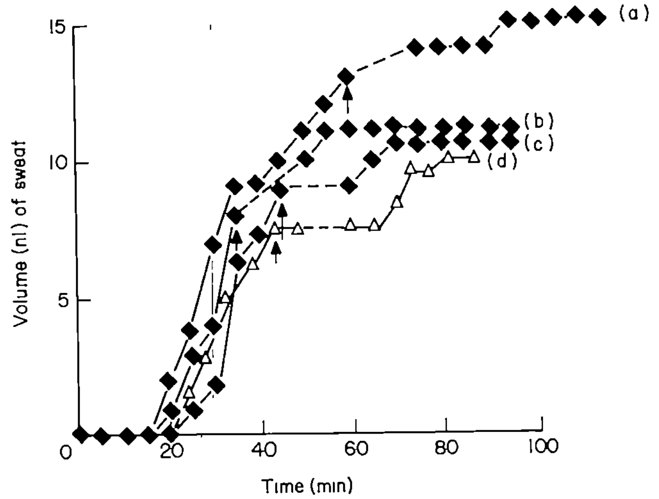

FIGURE 5. Effect of temperature drop (from 38°C to 4°C) on carbachol-induced sweat secretion *in vitro*. The temperature of the medium was changed at the time indicated by the arrows. At 15 minutes carbachol (3×10^{-5} M) was added to the bathing medium.

DISCUSSION

Theoretical as well as practical problems have always surrounded *in vivo* sweat gland study and this has impeded a more precise understanding of the mechanism of sweat secretion. It was therefore important to develop a new *in vitro* technique by which this problem could be studied in well-defined and controlled experimental conditions. Sato (1973) attempted to apply *in vitro* methods developed initially for the isolated kidney tubule (Burg *et al.*, 1966) to the monkey eccrine gland. Sato concluded that the monkey eccrine sweat gland was an ideal model for investigation of the mechanism of sweat secretion. Prompt and Quinton (1978) applied a similar technique to human glands in order to investigate the effects of calcium ions on the secretory process. Calcium is an important regulatory factor in many physiological systems, including fluid-transporting epithelia. There is good evidence that such regulatory factors are involved in normal secretory function (Thorn, 1974), and indirect evidence that they may be involved in secretory irregularities in disease states (Dearborn & Dorr, 1976; Rubin, 1970).

The results in this study are in agreement with those obtained for isolated monkey eccrine sweat glands (Sato, 1973, 1974, 1977) and rat glands (Sato & Sato, 1978). Approximately one-third of the dissected glands failed to secrete sweat following stimulation by methacholine or carbachol. In some, secretion stopped within half an hour following sweat induction. The probable explanation for this was the infliction of tissue injury during the dissection of the glands. An important question concerning the validity of this technique of *in vitro* sweat induction, was whether the fluid collected was secreted sweat or incubation medium that had leaked in. The following observations suggested the former: (a) no sweating was observed at 4°C; (b) no sweating was observed at 38°C prior to drug administration, (c) after drug administration active glands produced sweat within the following few minutes, (d) atropine administration inhibited sweat secretion, (e) calcium lack in the bathing medium inhibited sweat secretion, (f) sweat induction was being performed at zero hydrostatic pressure. Sweat rates obtained were lower than those reported by Sato and Sato (1978) of 2 nl/gland/minute using rat eccrine sweat glands. The present investigation also used specially designed apparatus which allowed the medium to be circulated continuously. This situation avoided forming of the medium which occurred during the gassing and mixing stages of the experiment (Michniak, 1980).

Further development of this technique is needed to improve the viability of the sweat glands but it appears to have great potential.

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Clinical, pharmacological and immunological aspects of delayed pressure urticaria

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SUMMARY

We studied the clinical features of thirty-two patients with delayed pressure urticaria, and special laboratory tests were performed in seven patients. Striking clinical features included a long duration of the disease (mean 6 years) and an elevated erythrocyte sedimentation rate in 71%, dermographism in 63% and a leukocytosis in 33% of the patients. There was prolongation of weals in response to histamine, compound 48/80, concanavalin A and NaCl. In some patients, histamine and chemotactic factor levels were increased in suction blisters over skin test and delayed pressure sites. In extracts from pressure weals, chemotactic activity was found for leukotriene B_4, its 20-ω-oxidation products and mono-HETEs. Studies of peripheral blood leukocytes revealed significantly increased intracellular histamine levels and increased release of histamine, and a trend to increased release of chemotactic activity from stimulated patient cells. The response of leukocytes to mitogens was normal.

We conclude that histamine plays a major role in the pathogenesis of PU. Arachidonate-derived chemotactic factors might account for the variably observed leukocytosis and the cellular infiltrate in lesions of pressure urticaria. Additional mediators must be involved in PU in order to explain the unique prolonged wealing response.

Pressure urticaria (PU) is characterized by the delayed onset of deep cutaneous swelling, erythema, pruritus or pain at sites of local pressure. Some patients also experience systemic symptoms such as fever, chills and malaise. The disease is particularly distressing to patients who perform manual labour since the swelling is not responsive to treatment with anti-allergic drugs other than corticosteroids (Warin & Champion, 1974).

The pathogenesis of PU is poorly understood. Since antihistamines do not influence the pressure weals, histamine probably plays no essential role in the evolution of the lesions. This does not, however, rule out the involvement of mast cells, as was suggested by the observation that tissue sites are refractory to the development of pressure lesions after prior depletion of mast cell stores by the mast cell degranulator, compound 48/80 (Ryan, Shim-Young & Turk,

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1968). Complement levels and protease inhibitors are normal in PU (Sussman, Harvey & Shocket, 1982; Doeglas & Bleumink, 1975), and defects in other mediator systems must therefore be looked for in order to explain the typical delayed weal formation after application of pressure.

We have studied the clinical features in thirty-two patients with PU, and this is the largest series of patients so far reported. In addition, the potential pathogenic role of histamine and the lipoxygenase products of arachidonate metabolism has been examined. Our data define the characteristic clinical features of PU and add further insights into the possible pathogenesis of the disease.

METHODS

The clinical characteristics of thirty-two patients with skin test-proven delayed PU (Illig & Kunick, 1969) were assessed by means of a questionnaire, and routine laboratory tests were performed. Seven of the patients, six men and one woman, agreed to participate in more detailed and extensive testing procedures and were admitted to hospital for this purpose. These latter patients suffered considerably from their PU and were therefore particularly keen to help in clarifying the pathogenesis. Three of these patients had a coexisting chronic idiopathic urticaria and dermographism.

Skin tests

Fifty μl of the following reagents, suspended in sterile physiological saline, were injected in duplicate intracutaneously on both sides of the back: histamine, 0·01%, compound 48/80, 0·01%, concanavalin A (Con A), 0·01% (all from Sigma, Munich, F.R.G.) and saline alone. Pressure was applied using 900 and 1800 g weights with a special device for 10 and 20 min over the back (Illig & Kunick, 1969). Skin test sites were examined immediately after removal of the weights and for up to 20 hours. Sizes of all weals were measured at their largest and smallest diameters for up to 6 hours. Results are expressed as the product of the two values obtained from each weal.

Suction blisters were applied over weals and normal skin with a Dermovac device, as previously described (Dierksmeier, Frosch & Czarnetzki, 1980). Blister fluid was boiled immediately after collection for 10 min and was then stored at −20°C until mediator assay. From one patient, several 2 mm punch biopsies were taken from normal and involved skin. The tissue was weighed, suspended in Hank's buffer, boiled for 10 min, and the tissue supernatant was assayed for chemotactic arachidonate metabolites by reverse-phase high-pressure liquid chromatography (RP-HPLC), as previously described (Grabbe *et al.*, 1984).

Mediator assay

Histamine was measured after the fluorimetric method of Shore, Burkhalter & Cohn (1959). Chemotaxis was performed in modified Boyden chambers, with guinea-pig eosinophils or human neutrophils as target cells (Czarnetzki, König & Lichtenstein, 1976).

Studies of blood leukocytes

Peripheral blood leukocytes were obtained from heparinized blood after dextran sedimentation, washed twice in Hank's buffer, adjusted to 1×10^7 cells/ml and incubated for 20 min, at 37°C in buffer alone or with the ionophore A23187 added (a gift from Dr Hamill, St Louis, U.S.A.) at 10^{-6}M. Cell pellets and cell supernatants were assayed for histamine and chemotactic activity.

The modulation of chemotactic activity was studied by pre-incubation of the cells for 10 min at 37°C with two cyclo-oxygenase inhibitors of arachidonate metabolism, aspirin (Bayer, Leverkusen, F.R.G.) from 10^{-4} to 10^{-8}M and indomethacin (Sigma, Munich, F.R.G.) from 10^{-6} to 10^{-8}M, and with three inhibitors of the lipoxygenase pathway, nordihydroguaretic acid, 10^{-5} to 10^{-6}M, benoxaprofen, 10^{-4} to 10^{-5}M (both from Dr M.Mardin, Wuppertal, F.R.G.) and compound BW 755C, 20 and 2 μg/ml (from Dr R.C.Flowers, London, U.K.) (for detailed methods, see Czarnetzki, 1983). The mitogenic responsiveness of lymphocytes was studied after a 3-day culture in the presence of various concentrations of Con A and phytohaemagglutinin (Czarnetzki & Macher, 1980).

Data are expressed as mean ± 1 s.d., and significance was tested by Student's *t*-test.

RESULTS

Table 1 summarizes the most important clinical features observed in the thirty-seven patients. The onset of the disease is generally within the 3rd to 5th decade of life, and it may persist for many years. After application of pressure, the deep-seated weals develop with a delay of 1–10 h and last for at most 30 h. More than half of the patients experience systemic symptoms such as malaise, fever and chills, and there is frequently an associated elevated erythrocyte sedimentation rate and a leukocytosis, but no eosinophilia. PU is not associated with an increased personal or family history of atopy, although dermographism or chronic urticaria often coexist.

In order to assess whether delayed wealing in PU is due to an abnormal mediator release or to an altered cutaneous responsiveness to the mediators involved, skin tests were performed with histamine and with two mast cell liberators, compound 48/80 and Con A. Early weals (10–15 min) in response to all four test substances did not differ between patients and controls (Fig. 1).

TABLE 1. Summary of important clinical and laboratory features of twelve female and twenty male patients with PU

	Mean ± 1 s.d.	Range
A. Age at onset (years)	34 ± 10	19–54
Duration (years)	6 ± 8	0·3–39
Delay until onset of lesions (h)	4·5 ± 2·0	1–10
Duration of lesions (h)	20 ± 8	2–30
	Percentage of all patients	
B. Systemic symptoms	58	
Leukocytosis (> 10,000/mm³)	33	
Eosinophilia (> 300/mm³)	0	
Elevated ESR	71	
Coexistent dermographism	63	
Coexistent chronic urticaria	31	
Reaction to parabens and analgesics	44	
Atopic personal history	6	
Atopic family history	13	

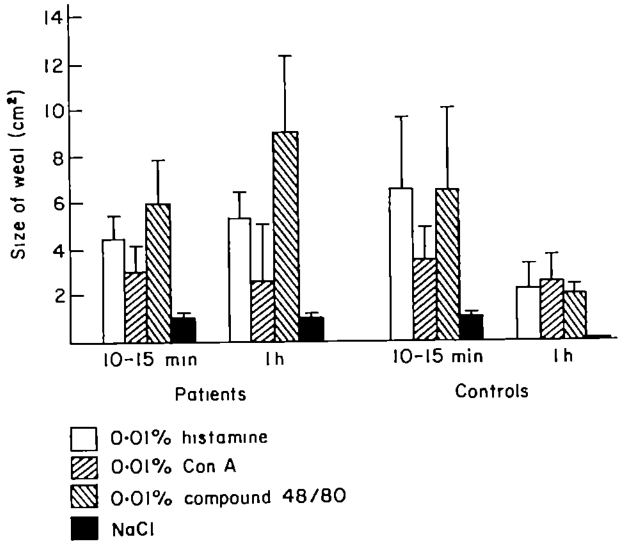

FIGURE 1. Reaction to intracutaneous injection of histamine, concanavalin A, compound 48/80 and NaCl. Sizes of weals were measured at 10–15 min and at 60 min. Differences between patients and controls are significant for histamine and compound 48/80 at 1 h only ($P < 0{\cdot}05$).

By 1 h, the histamine and compound 48/80 weals were however larger in the patient group ($P < 0{\cdot}05$). After 4 h, the sizes of Con A and NaCl weals also increased markedly while they resolved in controls at about this time. In a few PU patients, weals were still noted 3 days after injection.

The levels of mediators in suction blisters from different types of weals and in suction blisters from normal skin of patients and controls are shown in Figures 2 and 3. In PU patients, mean histamine levels were elevated at Con A and 48/80 injection sites and at pressure sites, compared with control sites (Fig. 2). The values differed widely however from patient to patient, and

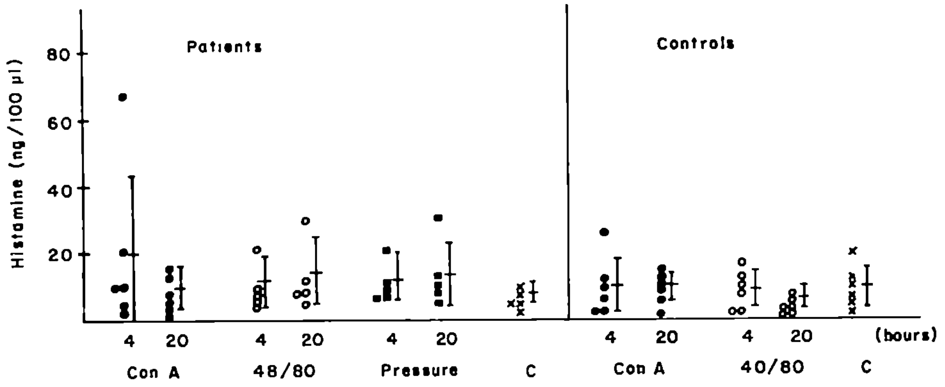

FIGURE 2. Scattergram of histamine levels in suction blisters over normal skin (C) and over 4 and 20 h old weals at injection and pressure sites (see Fig. 1). There were no significant differences.

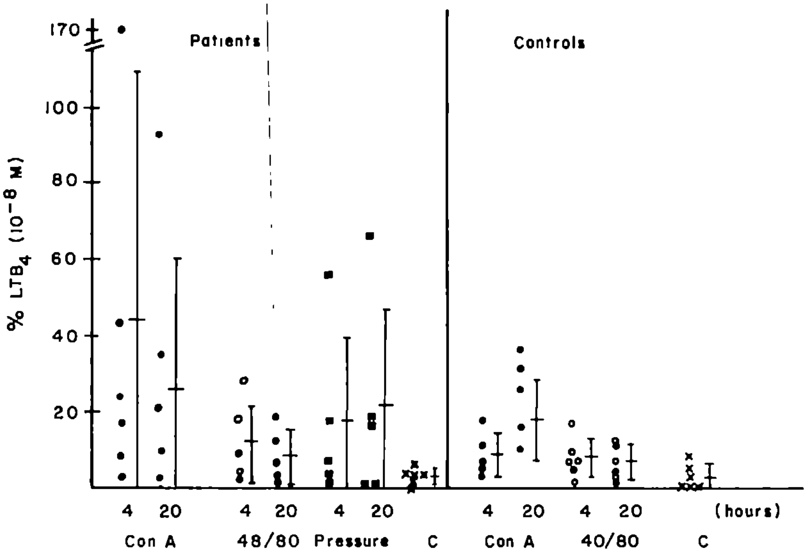

FIGURE 3. Chemotactic activity in blister fluid obtained as in Fig. 2. Results are expressed as % activity compared with the standard LTB_4, 10^{-8}M (100%). Because of the large scatter and the small numbers of subjects, the mean differences are not statistically different, but there is a trend to increased release of chemotactic mediators in patients.

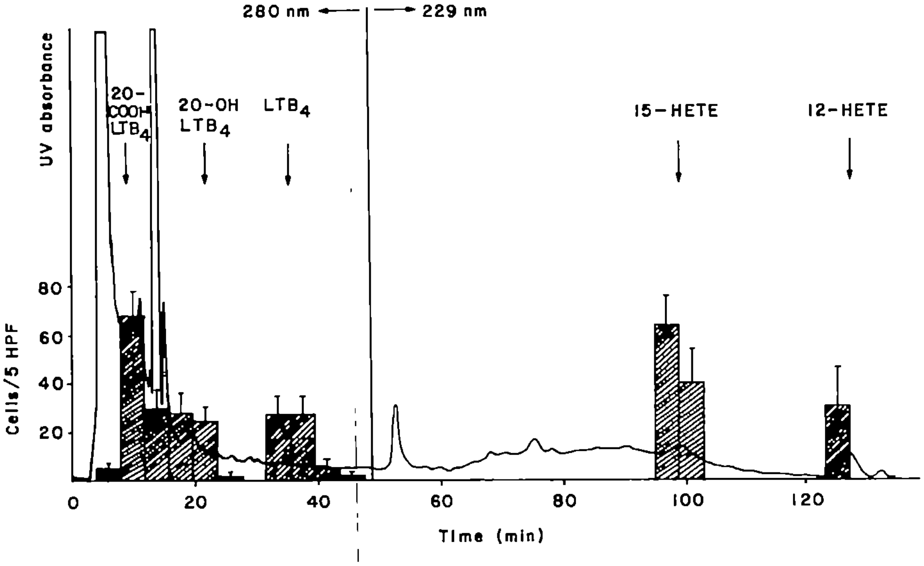

FIGURE 4. Elution profile of tissue extracts from PU weals on RP-HLPC. Absorbance is shown in arbitrary units on the ordinate and the elution time on the abscissa. Positions of chemotactic marker molecules are indicated by arrows. A number of unknown peaks are also noted. Chemotactic activity in the fractions is drawn in vertical bars and expressed as cells that have migrated through a chemotaxis filter in 5 high power microscopic fields (HPF).

TABLE 2. Mean ± 1 s.d. of total leukocyte histamine and of mediator release on stimulation of leukocytes with the ionophore A 23187, 10^{-6} M. Cellular histamine and % histamine release, but not the release of chemotactic activity, were significantly different in patients and controls

	Patients	Controls	*P* value
Total histamine (ng/2 × 10^6 cells)	75·8 ± 29·9	163·5 ± 135·6	<0.05
Histamine release (% of total cellular content)	32·3 ± 10·0	21·7 ± 6·4	<0.05
Release of chemotactic activity (% of LTB_4, 10^{-8} M)	136·8 ± 139·9	60·3 ± 44·8	>0.05

statistical significance was not reached. In control patients, mean histamine levels at injection sites did not differ from normal skin. Histamine levels were also examined in blisters over histamine weals and were elevated at 4 h (19 ng/100 μl, but not at 20 h (5 ng/100 μl) (not shown).

Figure 3 shows the levels of neutrophil chemotactic activity in the blister fluid suspensions. In contrast to the findings with histamine, levels were close to baseline in normal skin of patients and controls. Over Con A weals, they were markedly elevated at 4 and 20 h with patients and controls, while they remained low at sites of compound 48/80 weals in patients and controls. In three out of five patients, blister fluid contained elevated chemotactic activity at sites of pressure weals.

The chemical nature of the chemotactic activity in PU weals was assessed in tissue extracts of a 20 h lesion and of normal skin from the same patients. The elution profile (Fig. 4) was similar for the extracts from both sites, except that peaks for LTB_4 and the mono-HETEs were slightly higher in extracts of pressure weals. This corresponded to a 16% higher total chemotactic activity in the extracts of the lesions. Peaks of chemotactic activity were found at the marker positions for LTB_4 and its two ω-oxidation products, and at two positions that corresponded to 15- and 12-HETE (Fig. 4).

Since the defect in PU might not be restricted to the skin, peripheral blood leukocytes were examined for mediator content and release in patients and normal controls. Total histamine was found to be reduced by about 50% in patient leukocytes (Table 2), while release of histamine and of chemotactic activity was enhanced. No chemotactic activity was measured in unstimulated patient and control leukocytes. Aspirin and indomethacin enhanced release of chemotactic activity while benoxaprofen (10^{-4} M), nordihydroguaretic acid (10^{-4} M) and compound BW 755C (20 μg/ml) were totally inhibitory (not shown). No quantitative differences between patients and controls were noted with these measurements. Lymphocyte transformation tests also revealed no differences between patients and controls (data not shown).

DISCUSSION

Our data confirm that PU differs from other types of urticaria in several ways. The most outstanding clinical aspects are a leukocytosis and an elevated sedimentation rate which have otherwise been reported only in rare types of urticaria such as familial cold urticaria (Warin & Champion, 1974).

Histamine and compound 48/80 increased the size of weals at injection sites in PU patients at 1 h, and the same phenomenon was also noted after 4 h at NaCl and Con A sites, thus mimicking the kinetics of the patients' pressure weals. This finding suggests that the skin of patients is more reactive to mast cell derived mediators. On the other hand, one must suspect that any type of intracutaneous injection, even NaCl, will result in delayed cutaneous reactions, because of the mechanical pressure applied to the tissue by the injection of fluid. Leukocyte histamine levels and the release of this mediator from cells are increased in patients with PU (Table 2), and one may therefore suspect that changes in PU are not restricted to the skin. Histamine levels in blister fluid at sites of pressure weals are however only slightly raised (Fig. 2). This may be due to the fact that histaminases are released from infiltrating leukocytes at pressure sites which destroy mast cell derived histamine in the tissue. Such a mechanism is more likely since blister fluid over histamine injection sites showed elevated levels of this mediator after 4 h. Another conclusion to be drawn from the variable levels of histamine at pressure sites may be that histamine is not the primary mediator of pressure-induced wealing, as is also suggested by the ineffectiveness of antihistamine therapy.

The measurements of chemotactic activity over weals yielded relatively high levels in PU patients (Fig. 3), compared with the histamine values. However, the scatter in the data obtained from blister fluids was very high, and one can see only trends towards higher mean values in PU patients. There are several possible reasons for this variability of the data and the lack of statistical significance:

(1) Variations in the clinical disease spectrum. Patients with other coexisting urticarias had however no strikingly different mediator levels in their blister fluid.

(2) The low subject numbers.

(3) Variations inherent in the bioassay for chemotaxis.

(4) Variable levels of serum and leukocyte-derived enzymes in suction blister fluid and in the tissue. These enzymes might further metabolize and decrease the biological activity of the factors, as may also happen with histamine and histaminase.

(5) Destruction of the factors by oxidation.

Figure 3 shows nevertheless that in three out of five patients, levels of chemotactic factors were elevated in the blister fluid extracted from early and late pressure weals. Part of the chemotactic activity can be attributed to lipoxygenase-dependent arachidonate metabolites, as is suggested by the identification of these mediators in tissue extracts on RP-HLPC (Fig. 4). Leukotriene B_4, and less so its 20-ω-oxidation products and the mono-HETEs, make up the chemotactic activity derived from this pathway, and LTB_4 is one of the most potent chemo-attractants known (Goetzl, 1980; Czarnetzki & Grabbe, 1983).

The increased release of these chemotactic factors from tissue and blood leukocytes may explain the leukocyte influx at PU lesions and the blood leukocytosis which also varies from patient to patient and with time.

The data in Figure 3 also show that patient weals contain more chemotactic activity at Con A injection sites than controls. This fits again with the increased releasability of this same activity from many patients' peripheral leukocytes. With compound 48/80, a poor releaser of chemotactic activity, these changes are not evident.

Doeglas (1975) has made the intriguing observation that 43% of patients with PU have an intolerance to aspirin and related drugs. These drugs also modulate arachidonate metabolism, and they were therefore used to probe the sensitivity of patient leukocytes to modulation of their cyclo-oxygenase (prostaglandin) and lipoxygenase (leukotriene) pathways. Corticosteroids, the only therapeutic agents effective in PU, totally block arachidonate metabolism. The data showed no differences in the sensitivity of patient and control leukocytes to the modulators of arachidonate metabolism, so that a defect in the activity of these enzyme pathways seems unlikely in PU.

Besides arachidonate-derived chemotactic factors, it is quite likely that complement fragments or the high molecular weight neutrophil chemotactic factor in mast cell granules (Tannenbaum *et al.*, 1980) may also contribute to the chemotactic activity measured in blister fluid extracts. Lymphocyte derived chemotactic factors might be a fourth group of mediators involved. These latter agents are released from stimulated cells only after a lag period of 1–2 hours (Altman, 1978), and they can induce histamine secretion from mast cells (Thueson *et al.*, 1979). Although our data on the mitogenic responsiveness of lymphocytes show no abnormalities in patients with PU, their lymphocytes might respond to an antigen generated by pressure at skin sites, resulting in lymphokine production, leukocyte influx and mast cell mediator release. Whether such mechanisms are operative at pressure sites must be the object of future studies.

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Localized granuloma annulare is associated with insulin-dependent diabetes mellitus

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SUMMARY

The prevalence of diabetes mellitus in 557 patients with granuloma annulare (GA) has been assessed by a retrospective study, using age-matched population data. A definite association was confirmed between GA and insulin-dependent diabetes with 16 observed cases compared with 0·9 expected, and in all but one case the diabetes was associated with the localized form of GA. Two further cases developed insulin-dependent diabetes within 5 years of presentation with GA.

Several dermatological disorders occur in association with diabetes mellitus. Between 65% and 75% of cases of necrobiosis lipoidica are found to be diabetic (Hildebrand, Montgomery & Rynaerson, 1940) and others show impaired carbohydrate tolerance (Hill *et al.*, 1966). The relationship with granuloma annulare (GA) is less clear-cut and the evidence is conflicting. The clinical features of GA have been well documented (Beare & Cunliffe, 1979), and although there is little doubt of the diagnosis in most cases, the histological appearance is strikingly similar to necrobiosis lipoidica. Most reports provide no evidence of an association between the localized form of GA and either insulin-dependent diabetes (IDDM) or non-insulin dependent diabetes (NIDDM) (Gould *et al.*, 1976; Mobacken, Gisslen & Johannisson, 1970; Williamson & Dykes, 1972). Some reports suggest a relationship between an atypical generalized form of GA and 'maturity onset diabetes' or impaired carbohydrate tolerance. The latter observation is based upon the results of cortisone stress tests (Haim *et al.*, 1973; Haim, Friedman-Birnbaum & Shafrir, 1970; Hammond, Dyess & Castro, 1963), but the validity of this test for predicting the future development of diabetes is now questioned.

For this study we have examined retrospectively a large series of patients with GA, to analyse the prevalence of diabetes mellitus at the time of presentation and after follow-up. We have used age-matched population data from two sources in the interpretation of the results.

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METHODS

Patients with GA were identified from the indexing systems of several dermatology departments in East Anglia. The inclusion of cases into this study has been made largely on clinical grounds and only 10% had been subjected to histological confirmation. It was noted whether the patient was known to be diabetic (IDDM or NIDDM) at the time of diagnosis of GA. A simple questionnaire was sent to each patient's general practitioner asking whether the patient had developed diabetes mellitus since GA diagnosis and if so of what type. Data were also obtained on the age and sex structure of the group, site, type of GA lesions (e.g. localized or generalized) and other associated medical conditions.

RESULTS

Granuloma annulare

A total of 679 patients with granuloma annulare were included in the study and replies to the questionnaire were received from 557 patients (82%). Table 1 displays the age at presentation by sex. The female to male ratio in the total sample was 1·25:1, and in the follow-up sample 1·6:1. The time since initial presentation with GA ranged from 1 to 30 years. The age and sex structure accords with the published figures, in that the majority of cases (86%) occurred within the first three decades of life and only 16% occurred over the age of 40 years. Localized GA occurred in 511 cases (93%) and generalized GA in 38 (7%) (Table 2). This information was not available for eight patients. There was a tendency for the atypical generalized type to affect the older age group. The sites most frequently involved were hands and arms, and lesions were found in multiple sites in 130 patients.

TABLE 1. Age at presentation and sex of GA patients

Age group	Males	Females
0–24	124	176
25–44	62	121
45–64	21	42
65 and over	6	5
Total, all ages	213	344

TABLE 2. Distribution of GA by sex of patient (information on distribution not available for eight patients)

	Males	Females
Localized GA	195	316
Generalized GA	16	22
Total	211	338

TABLE 3. Observed and expected numbers of IDDM patients by age at presentation with GA

Age at GA presentation	Total sample	Observed IDDM	Expected IDDM (Green *et al.*, 1981)	Expected IDDM (Williams, 1982)
0–24	300	10	0·5 (P < 0·001)	0·3 (P < 0·001)
25–44	183	3	0·5 (P < 0·05)	0·5 (P < 0·03)
45–64	63	1	0·3 NS	0·1 NS
65 and over	11	2	0·1 (P < 0·01)	0·01 (P < 0·001)
Total, all ages	557	16	1·4	0·91

NS = not significant.

Diabetes

There were twenty-four patients (4%) with diabetes, comprising eighteen IDDM and six NIDDM. In all but two cases the diabetes was already present or was diagnosed at the time of presentation with GA. The two other patients both had IDDM which developed within 5 years after presentation with GA. As expected, the majority of the IDDM patients were found in the younger age groups, and all six NIDDM patients were over 45 years old at GA presentation. In all but one case the diabetes was found in association with the localized form of the disease; the one case of generalized GA was present in an IDDM patient. The expected numbers of diabetics in each age group in a population sample of this size were calculated from prevalence data from recently published English and Danish population surveys (Williams, 1982; Green *et al.*, 1981). The probabilities of obtaining the observed results were calculated by comparison with the appropriate distributions with these expected values as their means (Armitage, 1971). For the three youngest age groups the Poisson distribution was taken, and for the smaller sample of 65 years and over, the binomial distribution (Table 3). In the youngest age group there was a highly significant excess of IDDM patients. In the 25–44 and 65 and over age groups a statistically significant excess was also observed. No analysis was performed on the non-insulin dependent diabetics because of the small numbers involved. Also the incidence of diabetes in patients after GA diagnosis was not considered for the same reason. There were three patients with IDDM who subsequently developed further lesions more characteristic of necrobiosis lipoidica diabeticorum some 6 months, 18 months and 5 years later. No other diseases were found to be associated with granuloma annulare.

DISCUSSION

This is the first study of granuloma annulare to examine the prevalence of diabetes and compare it with an age-matched non-GA population. It is also the largest series of GA patients so far reported. The largest previous study on record is that of Wells and Smith (1963), who collected data on 115 patients, but unfortunately nearly half of their original 208 patients were lost to follow-up. In the 115 patients they noted three with diabetes mellitus, but the type was not specified. The results of the present survey conform broadly with the published data for the age of onset and the sites affected. The sex ratio of 1·25 : 1 (female to male), however, is lower than that quoted by other groups of 2·2 : 1, and the reason for this difference is not known.

Most dermatologists are confident in their ability to differentiate granuloma annulare from necrobiosis lipoidica, but it is recognized that in a small number some clinical doubt may exist. It is also a fact that some clinical and histological overlap occurs and the two conditions may coexist (Burton, 1977). As noted earlier, only 10% of cases included had been confirmed histologically. Reference to thirty cases of necrobiosis lipoidica taken from the same indexing systems did not reveal any cases where the original diagnosis had to be modified on clinical or histological grounds. There are major histological similarities between granuloma annulare and necrobiosis lipoidica (Wood & Beerman, 1960), and it is this that has given rise to the speculation of a possible link with diabetes mellitus. In necrobiosis lipoidica a microangiopathy is found similar to that seen with diabetes, and a PAS-staining glycoprotein is present within the walls of small vessels. A similar change has been observed also in some lesions of granuloma annulare of the generalized type in the presence of maturity onset diabetes. An increased level of factor VIII-related antigen has recently been reported in the vessels of patients with generalized lesions of granuloma annulare (Majewski *et al.*, 1982). Factor VIII-related antigen is also elevated in the blood vessels of patients with diabetic angiopathy.

In a study of this kind various sources of bias may distort the results. Loss to follow-up is a major factor but the proportion of patients for whom incomplete data were obtained is small in this study. Also, diabetics are more likely to be seen regularly at hospital clinics and cross-referral to other clinics is a frequent occurrence. This would tend to increase the number of diabetics in such a series if non-diabetics with GA were more likely to go unreferred. Analysis of the source of referral did not indicate that this bias was present in this study since few (two diabetics only) were referred from hospital clinics and a search for other chronic conditions (asthma, rheumatoid arthritis) which are also subject to medical follow-up did not reveal an excess of cases. The possibility that diabetic patients with GA would be referred to a dermatologist by the general practitioner more often than non-diabetics with GA could not be tested.

The results of this study leave little doubt that there is an association of granuloma annulare with IDDM, although the reason for this association is still obscure. There is a recognized association of insulin-dependent diabetes with the HLA system, particularly with the haplotypes A1-B8-DR3-D3 and A2-B15-DR4-D4. Andersen and Verdich (1979) were able to demonstrate an increased frequency of B8 in patients with localized GA lesions, and, in another group, Bw15 (now designated B15) with the generalized type of granuloma annulare. Are patients with granuloma annulare more at risk of developing diabetes mellitus? Despite over half of the patients having a follow-up of 10 years or more, this question can still not be answered with certainty. The fact that only two patients developed IDDM and both within 5 years of the diagnosis makes it unlikely.

The question of a relationship with NIDDM also cannot be satisfactorily answered. The results do not support a relationship with the generalized form but there were six NIDDM patients out of a total of 88 patients over 40 years old. It is tempting to speculate that an association does exist. That four of these patients were diagnosed as diabetic at the time of presentation to the dermatologist and that the condition may improve with the control of the diabetes (Haim *et al.*, 1970), lends some weight to this suggestion. It would be difficult to collect a sufficiently large series of elderly GA patients to allow this to be adequately tested.

What should one do therefore when confronted with a young patient with granuloma annulare? Our results suggest that a family history and urine analysis or preferably a blood glucose is all that is required, as these simple tests will pick up most if not all of the at-risk patients. This appears to be so even for those patients who go on having recurrent lesions over many years. The sudden onset in the elderly should arouse one's suspicions that there may be an underlying impairment of glucose metabolism.

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Cutaneous manifestations of Gaucher disease

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SUMMARY

We reviewed the cutaneous manifestations of type I non-neuropathic Gaucher disease in fifty South African patients. Characteristic changes such as diffuse pigmentation, easy tanning and flitting brown macules were found to be useful pointers to the diagnosis. Other cutaneous changes secondary to infiltration of the marrow and liver were common and important findings.

Gaucher disease is a heterogeneous inherited disorder of glycosphingolipid metabolism characterized by deficiency of the lysosomal hydrolase, acid-beta-glucosidase (Brady, Kanfer & Shapiro, 1965). The most common form is type I, chronic, adult or non-neuronopathic Gaucher disease, which occurs with maximal prevalence in individuals of Ashkenazi-Jewish ancestry (Frederickson & Sloan, 1972). In this disorder, which is inherited as an autosomal recessive, the main clinical features are splenomegaly, dyshaemopoeisis and orthopaedic sequelae of bone marrow infiltration. The clinical course is progressive, but fluctuates; survival to old age is not unusual.

A variety of skin manifestations have been reported as being common in individuals with type I Gaucher disease (Reich, Seife & Kessler, 1951), but it is questionable whether these are all genuine components of the condition. In order to elucidate this problem we have analysed the cutaneous changes in fifty affected South Africans whom we have investigated and managed since 1972. Our findings are discussed in this paper and contrasted with previous reports in the literature.

METHODS

During the past decade we have conducted a comprehensive investigation into type I Gaucher disease in Southern Africa. In this period we have examined forty affected adults of Ashkenazi-Jewish stock, together with ten members of the Afrikaner community. In each instance the diagnosis was confirmed biochemically. The clinical and genetic manifestations in these patients have been presented and discussed in detail elsewhere (Goldblatt, Sacks &

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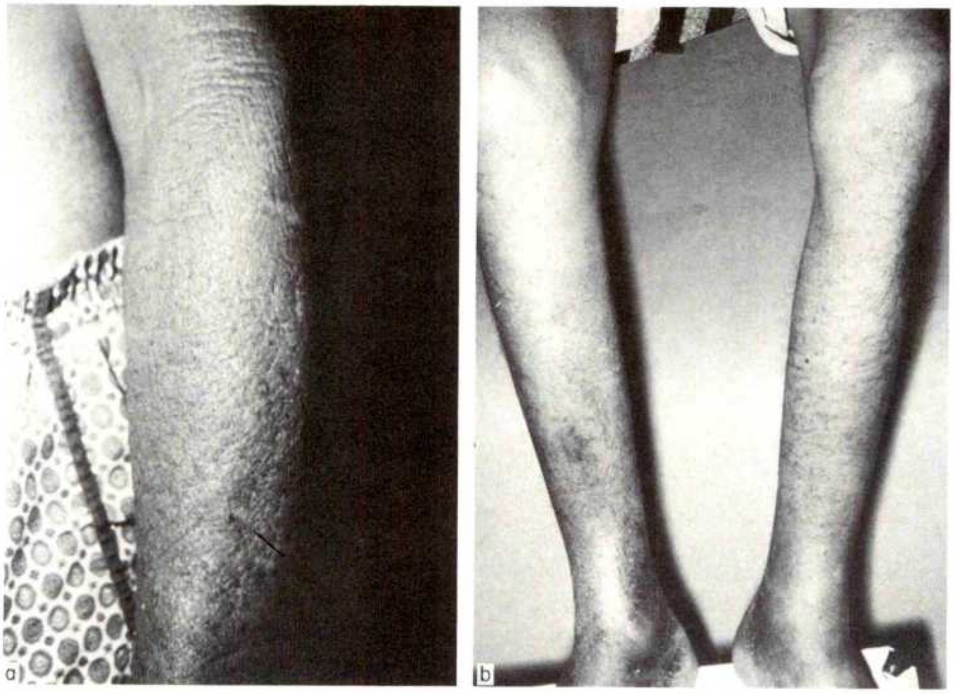

FIGURE 1. Diffuse hyperpigmentation and brown macules in Gaucher disease.

Beighton, 1978; Goldblatt & Beighton, 1979a,b, 1982). The skin changes in these patients were documented, and the analysis of this accumulated data forms the subject of this paper.

RESULTS

The abnormalities which were detected in the fifty patients are summarized below.

Diffuse pigmentation. Twenty-five patients (50%) had diffuse brown or yellow-brown hyperpigmentation with no specific peripheral localization (Fig. 1a,b).

Tanning. Twenty of the affected individuals (40%) described the phenomenon of 'easy tanning' and stated that they became deeply pigmented even in winter after minimal exposure to the sun.

Pigmented macules. Fifteen (30%) had non-specific brown macules which tended to be flitting and unstable. One patient described an exacerbation of these macules which occurred during an acute osseous crisis and was anatomically related to the affected bone.

Secondary manifestations. The secondary effects of Gaucher disease in the liver and bone marrow which lead to skin changes, including purpura, ecchymoses, pallor and rarely jaundice, were seen in the vast majority of affected individuals at various stages of their disease. Ten (20%) had telangiectasia (spider naevi) which were especially evident over the arms, chest and face. Two of these patients had markedly enlarged livers, but there were no other clinical or biochemical signs of hepatic decompensation.

None of our patients had streaky pigmentation, pigmentation which was maximal on the shins, a malar flush or haemorrhagic furunculosis, all of which have been reported as common features of Gaucher disease (see below).

DISCUSSION

The skin manifestations of Gaucher disease can be broadly classified into two main groups on the basis of:

(i) the primary effect of Gaucher disease on the skin;

(ii) the secondary skin effects resulting from Gaucher infiltration, especially of bone marrow, liver and spleen, with resultant dysfunction of the affected organ.

'Classical' descriptions of dermal stigmata of Gaucher disease are found in many older reviews of the condition (Reich *et al.*, 1951). These abnormalities include:

(i) chloasma-like patches of streaky pigmentation of non-specific distribution (Bloem, Groen & Postma, 1936);

(ii) peripheral and symmetrical hyperpigmentation which is described as extending from just below the knees to a sharply defined lower margin just below the ankles. This may be glossy, scaly and ulcerated (Bloem *et al.*, 1936; Groen & Garrer, 1948);

(iii) the rare occurrence of a peculiar malar flush (Bloem *et al.*, 1936; Groen & Garrer, 1948);

(iv) haemorrhagic furunculosis of the extremities with residual black pigmented scars (Reich *et al.*, 1951).

It is noteworthy that none of the above-mentioned changes were present in any of the individuals in our series. The reason for this discrepancy is uncertain; possible explanations include intercurrent disease, heterogeneity and the natural propensity for the perpetuation of misconceptions in the literature.

The most significant dermatological features in our patients were the generalized 'yellow-bronze' hyperpigmentation and the subjective description of easy tanning. The only practical importance of this characteristic skin appearance is its aid in the clinical diagnosis of Gaucher disease, and it requires no special management. The generalized nature of the hyperpigmentation which we observed possibly reflects the frequent exposure to the sun experienced by the South African patients.

Pick (1933) postulated that the skin pigmentation might be a manifestation of an underlying haemochromatosis. Furthermore, Wechsler and Gustafson (1940) found the dermal pigment was melanin or a melanin derivative which is also present in haemochromatosis. Gaucher cells are reported to affect iron metabolism related to erythropoiesis (Lee, Balcerzak & Westerman 1967; Van Slyck, Waldmann & Rebuck, 1974) and theoretically, Gaucher cell infiltration might result in the abnormal deposition of iron in the skin. Intradermal iron reduces the inhibitory effect of the glutathione radical on the tyrosinase enzyme. The consequent enhanced tyrosinase activity results in increased melanin formation by accelerating conversion of the precursor tyrosine to Dopa. This hypothetical model could account for the dermal pigmentation in affected individuals.

There is no obvious explanation for the high incidence of telangiectasia in our patients. In particular, these changes were not associated with any clinical or biochemical evidence of hepatic decompensation but it is possible, however, that they represent a very early effect of hepatic dysfunction. It was also notable that non-specific brown macules were present in almost a third of the patients, but their significance is uncertain.

The skin involvement which we observed was entirely benign and neither caused clinical problems nor necessitated any form of therapy. However, the secondary skin changes are important indicators of haematological or hepatic complications. On the basis of our experience we consider that the recognition of the dermatological features represents a useful adjunct in the diagnosis and management of type I Gaucher disease.

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Case Reports

Thirteen British cases of erythema chronicum migrans, a spirochaetal disease

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SUMMARY

Thirteen British cases of erythema chronicum migrans are reported. An antibody to the Lyme disease spirochaete was found in three of five recent cases, but Lyme disease was not seen. The distribution and life cycle of the tick *Ixodes ricinus* is described and related to the occurrence of erythema chronicum migrans in Great Britain.

Erythema chronicum migrans (ECM) is a distinctive skin condition that has been known in Northern Europe since its recognition by Afzelius in 1910. It has recently been reported in the United States of America (Scrimenti, 1970) and in the United Kingdom (Obasi, 1977; Goldin *et al.*, 1978). The condition is believed to be infectious and to be transmitted by insects. Although in most cases the vector is a tick, cases of ECM have occurred following a mosquito bite (Hard, 1966) and a thorn injury (Bessiere, 1966). The typical history is of an insect bite that subsides to leave a slowly enlarging ring of erythema. The lesions are usually solitary but multiple lesions do occur, and in many cases they are so unobtrusive that they may escape notice for months.

The association with an acute arthritis was observed in a number of cases from the town of Lyme in Connecticut (Steere *et al.*, 1977a). This association was initially called Lyme arthritis but was amended to Lyme disease in 1980 with the realization that the arthritis was only part of the clinical picture. This new disease prompted research into the aetiology of ECM, and recently spirochaetes have been isolated from several patients with Lyme disease (Steere *et al.*, 1983; Benach, Bosher & Hanrahan, 1983).

This paper describes thirteen cases of erythema chronicum migrans seen in the Cambridge Dermatology Department, and highlights some factors which may be responsible for the case-clustering around the Thetford area of East Anglia. Evidence is presented supporting the role of spirochaetes in the aetiology of ECM in Great Britain.

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TABLE 1. Cases of erythema chronicum migrans seen in Cambridge 1968–1983. Antibody titres to the Lyme disease spirochaete are shown for patients 8–13

Patient No.	Duration of lesion prior to presentation	Bite history and site	Treatment	Place where bitten	Season	Time to clear	Date of illness	Antibody titre to spirochaete
1	2 weeks	+ leg	None	Thetford	July	8 weeks		
2	Unknown	+ leg	Penicillin	N. Europe?	June	10 days		
3	Unknown	− leg	None	Thetford	June	6 weeks		
4	4 months	+ leg	Penicillin	Thetford	June	10 days		
5	2 months	+ leg	Penicillin	N. Europe	July	No follow up		
6	1 month	+ leg	Penicillin	Nr. Thetford	June	7 days		
7	6 months	− leg	Penicillin	Thetford	July	7 days		
8	6 months	+ leg	Penicillin	Thetford	July	7 days	1978	Neg.
9	4 months	+ leg	Tetracycline	? Sweden	June	7 days	1978	Neg.
10	9 months	+ leg	Penicillin	Nr. Cambridge	June	10 days	1979	1:64
11	4 months	+ chest	Penicillin	Thetford	July	7 days	—	ND
12	6 months	+ leg	Penicillin	Thetford	July	7 days	1981	1:64
13	3 months	+ leg	None	Thetford	July	8 weeks	1982	1:256

CASE REPORTS

Thirteen patients have been seen over a 15-year period with ECM (Table 1). Ten of them had definitely contracted the illness in England but three gave a history of travel abroad around the time of onset of symptoms. Nine patients gave a definite history of an insect bite preceding the onset but in only two was this firmly attributed to a tick. In all but one case the lesions began on the lower limbs and all were solitary. Those patients who recalled an insect bite developed an irritable primary reaction followed by a faintly erythematous, slowly enlarging ring which in many cases extended from the ankle to the groin. The zones of erythema showed minimal induration and sometimes scaling in a band 2 to 3 cm wide, leaving central clearing behind the advancing rim of erythema. The size of the rings corresponded to the length of the history. None of the patients developed systemic symptoms at the onset or at any time during the course of the lesions.

Histology was assessed from one patient from the erythematous zone which demonstrated a scant perivascular mononuclear cell infiltrate which was predominantly lymphocytic. The Weil–Felix test was performed on three patients' sera with negative results. Once the diagnosis was established the patients were treated with penicillin V and in one case with oxytetracycline. This produced resolution in all patients within 7–10 days. One patient was lost to follow-up and two were not given antibiotics. Those untreated took a further 6–8 weeks for their lesions to disappear. A follow-up study on nine patients over a period of 1 to 10 years post-ECM has not revealed the development of any sequelae.

Convalescent sera were obtained from the five most recent cases and were examined by indirect immunofluorescence for the presence of antibodies to the Lyme disease spirochaete (kindly performed by Dr W.Burgdorfer at the Rocky Mountain Laboratories, Hamilton, Montana, U.S.A.). The spirochaete used in this investigation was obtained and cultured from an American patient with Lyme disease (human isolate R10). A thin film of spirochaetes was air-dried on a glass slide and incubated with the patients' serum. The second incubation employed a goat anti-human fluorescein isothiocyanate (FITC) labelled IgG. Examination was carried out under ultraviolet irradiation. Positive and negative controls were done for each patient. For further details see Burgdorfer *et al.* (1982).

RESULTS

Three patients had positive serology to the Lyme disease spirochaete detected by indirect immunofluorescence (Table 1). The two patients with negative results contracted ECM 6 years ago, whilst the highest titres were found in the patient with the most recent illness.

DISCUSSION

These thirteen cases of ECM emphasize the relatively benign course of the illness in British patients, and show that rapid clearance of the lesions occurs with penicillin. The accompanying symptoms are usually minor and include headache, malaise and nausea. Sometimes there may be a regional lymphadenitis. Meningitis was reported as a complication of ECM by Hellerstrom (1930), but in this and subsequent reports no organisms were isolated.

Lyme disease is a systemic illness that begins with ECM and is followed by either cardiac, neurological or arthritic symptoms days or weeks later. In addition to the constitutional symptoms of malaise, headache and nausea there may be an acute arthritis, cardiac arrythmias and mycocarditis (Steere, Batsford & Weinberg, 1980), and cranial nerve palsies or meningoencephalitis (Reik *et al.*, 1979). ECM may occur in the absence of any systemic sequelae. In one series of Lyme disease (Steere *et al.*, 1979), 10% of the patients who developed an acute oligoarthritis subsequently developed a chronic seronegative arthritis of the large joints. Immune complexes can be demonstrated in the serum and synovial fluid of patients with active Lyme disease. An association with the HLA-DR2 histocompatibility antigen has been found (Steere *et al.*, 1977a). Since the outbreak of Lyme disease in America, there have been case reports from Europe of both arthritis and neurological manifestations complicating ECM (Mallecourt, Landureau & Wirth, 1982; Gerster *et al.*, 1981).

The response to antibiotics suggests an infective agent as the cause of ECM and in 1948 Lennhoff reported that he had isolated spirochaetes from lesional tissue, but this could not be confirmed (Lennhoff, 1948). The direct transmission of ECM was later achieved by Binder (Binder, Doepfmer & Hornstein, 1955) using lesional tissue as the inoculum. The recent isolation of spirochaetes from the gut of the tick *Ixodes dammini* (Burgdorfer *et al.*, 1982) in an area endemic for Lyme disease supports Lennoff's original theory. This same spirochaete has been cultured from skin lesions (Berger, Clemmensen & Ackerman, 1983), blood and cerebrospinal fluid (Steere *et al.*, 1983) in a small number of patients. Elevated titres of anti-spirochaetal antibodies may be found in most patients with acute Lyme disease and also in the convalescent sera up to 3 years later. The same antibody to the Lyme disease spirochaete has been demonstrated in three of our patients, supporting the role of spirochaetes in the aetiology of ECM in Great Britain. An identical spirochaete has been found in *Ixodes ricinus* in Switzerland. More recently the spirochaete has been demonstrated in histological sections using the Warthin–Starry silver stain (Berger *et al.*, 1983), and by electron microscopy (Waldo & Sidhu, 1983).

There are twenty species of ticks in Great Britain mainly of the genus *Ixodes*, but *I. ricinus* is the most widely distributed (McLeod, 1962) (Fig. 1). Most of the tick life cycle is spent within the ground vegetation, but there is a twice-yearly period of activity when the larva, nymphs and adult ticks leave the ground vegetation to feed on their hosts. This feeding activity occurs in the

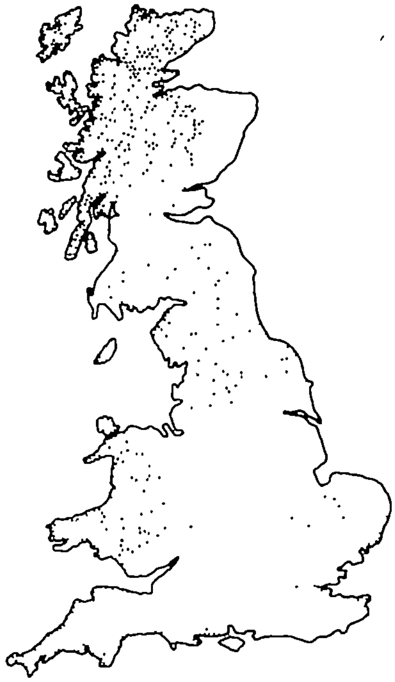

FIGURE 1. Distribution of *I. ricinus* in Great Britain (McLeod, 1962).

early summer and autumn. It is maximal in early summer and this accounts for the high incidence of cases of ECM in June and July. It is estimated that in Scotland approximately 95% of the tick population adopt the sheep as their principal host, whereas in East Anglia their hosts are mainly small mammals. Ticks can usually be found in woodland, scrub and moorland but their survival is dependent on the host density. Rough grazing and pasture land is less suitable because although the hosts are numerous the vegetation is continually being eaten away.

Tick bites are common in farming communities, so the paucity of recorded cases in other regions and the apparent case clustering within a small area of East Anglia is unexpected. A postal survey has shown that cases do occur elsewhere, in particular the Highlands of Scotland and Northern Ireland. Those cases reported by Goldin *et al.* (1978) form part of this present series. Most of our patients came from the Thetford area or had had recent contact with this area. Thetford Forest and Thetford Chase are popular recreational sites and ticks are known to be numerous in this area. It seems plausible to suggest that the incidence of ECM is proportional to both tick density and the prevalence of parasitism by the spirochaete. The method of parasite transmission is unknown. That ECM is not widespread in Great Britain suggests that spread may be limited by the host. In America new cases of Lyme disease have rapidly occurred in the neighbouring states, perhaps transmitted by the white-tailed deer, a common host capable of a wide dissemination of the tick or spirochaete (Spielman, 1976).

It is surprising that Lyme disease has not been seen in Britain. The infecting spirochaete may be different, but the finding of antibodies to the Lyme disease spirochaete and the isolation of an identical spirochaete in Switzerland suggests that this is not the case. The expression of Lyme disease with the HLA-DR2 antigen may be important. The diagnosis of erythema chronicum migrans may now be confirmed by indirect immunofluorescence using acute or convalescent sera.

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Ehlers–Danlos syndrome type IV mimicking non-accidental injury in a child

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SUMMARY

This case report describes a child in whom non-accidental injury was initially suspected, but who was later shown to have autosomal dominant Ehlers–Danlos syndrome (EDS) type IV. Her mother and brother were also type III collagen deficient. This syndrome may be commoner than the small number of reported cases suggested and may sometimes explain previously undiagnosed easy bruising. The importance of recognizing this syndrome is stressed as it may save a great deal of embarrassment, frustration and anger for the family wrongly suspected of causing injury to their child.

CASE REPORT

A 5-year-old girl was admitted to hospital at the request of her general practitioner as a suspected case of non-accidental injury ('battering') because of extensive bruising on her limbs and trunk. There were no haematological abnormalities and she seemed otherwise to be a fit, happy child. She was discharged the next day and a full case conference was later held. The case conference was inconclusive, but on subsequent review in the Outpatient Department it was felt that she was suffering from an inherited defect of connective tissue.

She was found to have bruising on the limbs and trunk, and poorly healed scars on her legs. Her skin had a transparent quality and the superficial venous system could be easily seen on her trunk. She had large brown eyes, a thin nose and thin lips (Fig. 1a). The skin on the dorsum of her hands was thin and slightly wrinkled (Fig. 1b).

Her mother and only brother had similar changes. In addition her mother showed marked changes of premature ageing of the skin of the face, hands and feet (Fig. 2). The mother, now aged 36 years, had suffered a third degree tear of the perineum on delivery of her first child and also antepartum and postpartum haemorrhages, both requiring transfusion. All three members of the family showed type III collagen deficiency diagnostic of EDS IV (Fig. 3).

DISCUSSION

At least nine different types of EDS can now be identified on clinical and biochemical grounds.

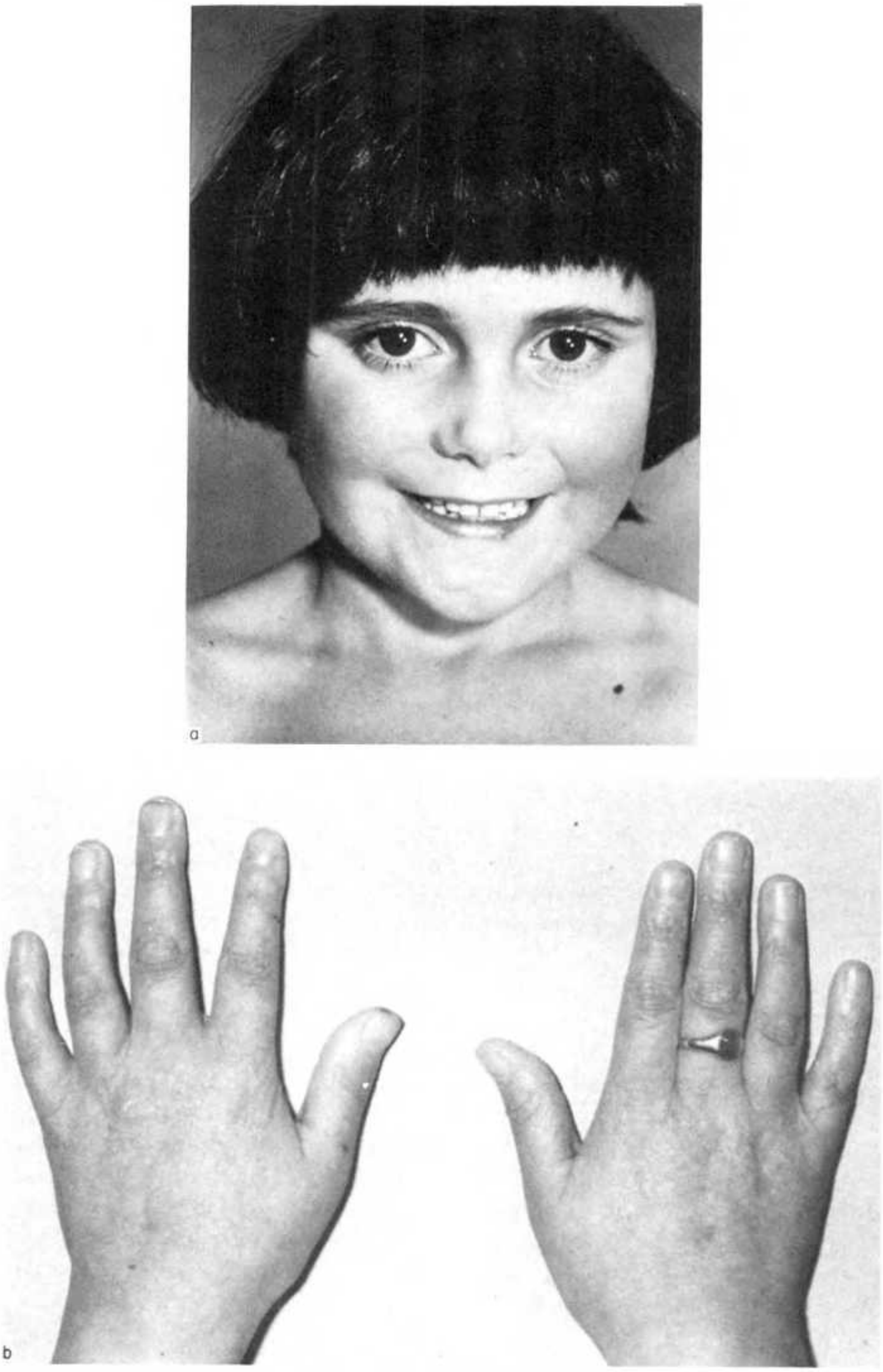

FIGURE 1. Face and hands of child with EDS IV.

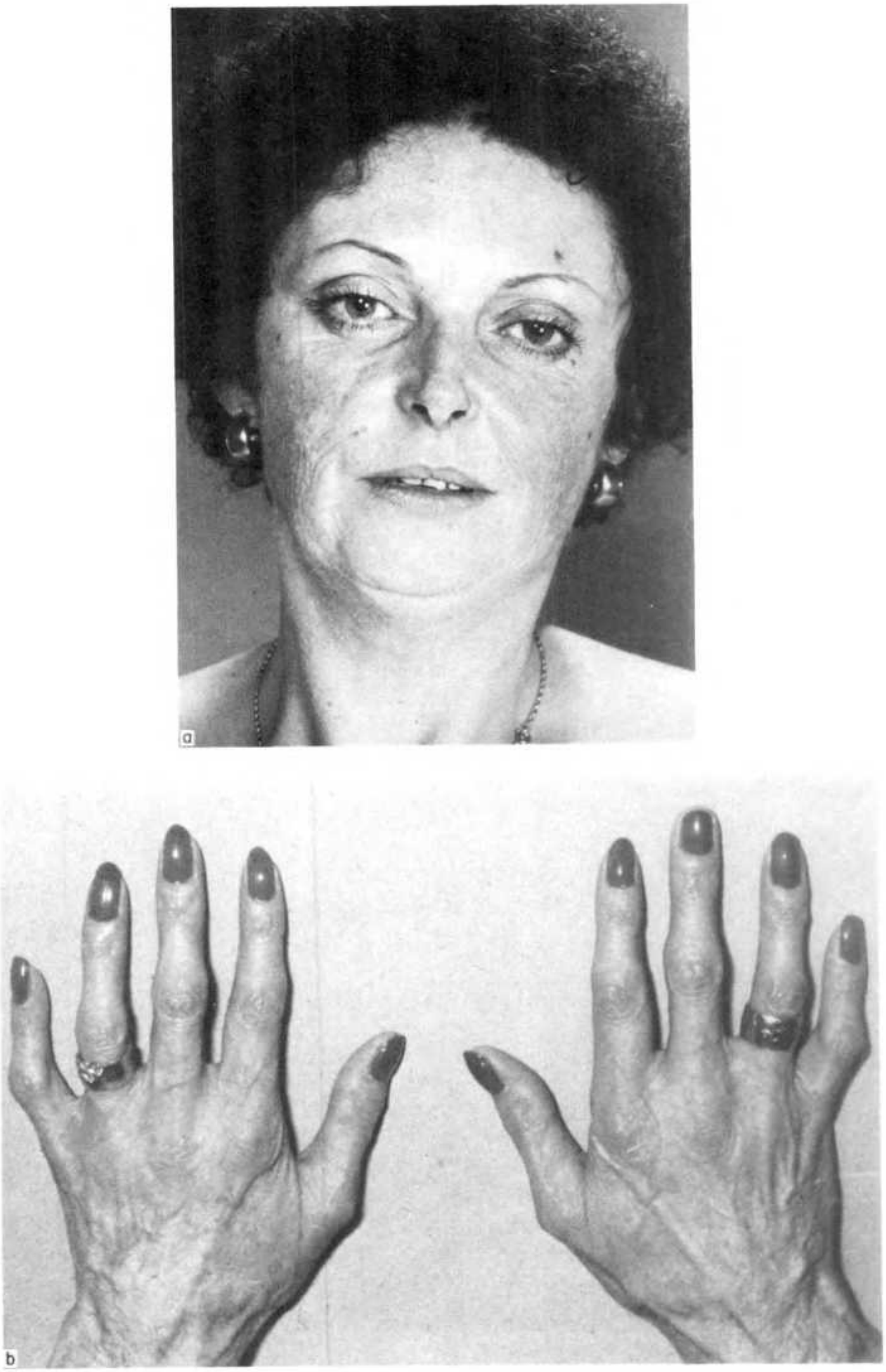

FIGURE 2. Face and hands of affected mother. Note prominent eyes, thin nose and prematurely wrinkled hands.

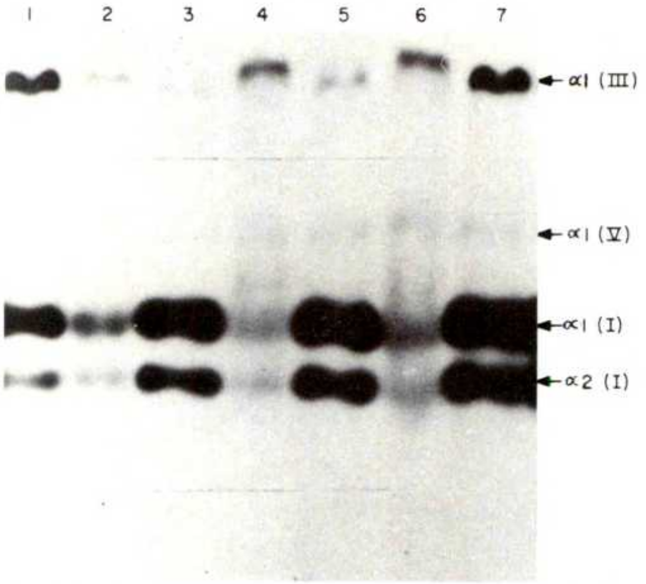

FIGURE 3. Electrofluoretogram of ^{14}C-labelled collagens produced on fibroblast culture. Type III collagens α1(III) have been separated from type I [α1(I) and α2(I)] by interrupted reduction. Tracks 1 & 2; Medium and intracellular collagens from controls. Tracks 3 & 4, 5 & 6 are from affected mother and child. Track 7; Medium collagen from controls. The affected individuals show the reverse of normal ratios with more intracellular type III retention which is consequently poorly exported with the cell medium.

EDS IV is itself a heterogeneous disorder. In 1975 we discovered a specific deficiency of type III collagen in tissues and cultured fibroblasts of patients with EDS IV (Pope *et al.*, 1975) and now recognize at least three variants within this group (Pope *et al.*, 1980). In the classical autosomal recessive form, death from aortic rupture in the third decade is common.

Clinical features include thin facies, prominent eyes, pinched nose, easy bruising and prematurely aged skin of the face and extremities (acrogeria). A milder atypical autosomal dominant form shows similar clinical features which are less marked and major vessel catastrophe is not a feature. The changes affecting the skin are subtle and may be easily overlooked.

Many older children with soft tissue injuries are now being diagnosed as cases of mild non-accidental injury, but the risk of false diagnosis if bruising occurs alone is very real and may cause great injustice to the suspected family (Jenkins & Gray, 1983). In our patient, establishing a specific diagnosis removed the social and emotional stigmata of being wrongly suspected of causing non-accidental injury.

The possibility of a type III collagen defect should be considered in all patients with a history of easy bruising without a haematological disorder. The importance of establishing the correct diagnosis may have important implications, not only in allaying any doubts regarding excessive violence as a cause of bruising, but also regarding prognosis and future genetic counselling.

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Comment

HISTOCOMPATIBILITY ANTIGENS (HLA) IN DERMATOLOGY

That genetic markers can indicate a predisposition to disease was suggested in the early 1950s by the finding that patients with duodenal ulcers had an increased incidence of blood group O and patients with gastric carcinoma had an increased incidence of blood group A (Aird *et al.*, 1953, 1954). The strength of the association was weak and a stronger predictive marker was needed. This need seemed to be filled by study of the HLA system, which had developed out of the necessity to find a way of matching donors and recipients in the transplantation of tissues. Up to March 1983, 611 papers have been published on HLA and skin and related diseases, but many of these have been based on small numbers or inadequate statistics. The literature up to 1978 has been reviewed by Ozawa, Ohkido and Kimiyoshi (1981).

Initially the antigens fell into four series, three of which, HLA-A, -B and -C, were serologically defined, and the fourth, HLA-D, lymphocyte defined. More recently, a series of antigens has been serologically defined on lymphocytes of B-cell origin which are closely related to the D locus, and are called DR antigens. It is likely that immune response and immune suppression genes occur in the DR region of the HLA complex. In 1979 there were seventeen A, twenty-seven B, seven C, eleven D and seven DR determinants and at that time it was calculated (Bodmer, 1980) that these could give rise to 300 million genetically different individuals and 30 million distinguishable combinations of antigens.

No HLA-disease association is absolute. The association of ankylosing spondylitis with B27 is most striking, but a few patients with ankylosing spondylitis are B27 negative, and many people with B27 do not get ankylosing spondylitis. Many explanations have been proposed to account for these contradictions. It is likely that non-HLA genes are also predisposing factors and that environmental factors such as infection may be important. Moreover, diseases are not necessarily homogeneous and HLA factors may be related only to certain clinical, pathological or immunological subsets of a disease. Resistance to a disease may be just as important as susceptibility. For example, it is known that HLA is associated with prognosis in Hodgkin's disease (Falk & Osoba, 1971) and acute leukaemia (Lawler *et al.*, 1974) and, in our experience, with systemic sclerosis.

Diseases seem to fall into two distinct groups in their relation to HLA types (Bodmer, 1980). The first group includes disorders considered to be autoimmune (I prefer the term autoaggressive) or have immunological features and these are usually associated with DR3 or B8. DR3 is in linkage disequilibrium with B8 and it is likely that the primary association is with DR3 rather than B8. Diseases in the second group are associated with an antigen, or antigens, of the HLA-A, -B or -C series, the most definite example of which is the relationship between ankylosing spondylitis and B27. It is important to realize that some associations may be primary and others secondary. For example, psoriasis is the only disease at present known to be associated with the C series. The association of psoriasis with CW6 is probably the primary association, and this induces a number of secondary associations such as B17, B13 and B37 known to be associated with CW6 in normal subjects. It is thought that CW6 in psoriasis may

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Based on a paper given to the Course on Progress in the Biological Sciences in Relation to Dermatology held at Churchill College, Cambridge from 26 to 30 September 1983.

involve non-immune mechanisms (Bodmer, 1980), as there is some evidence that genes can control non-immune cell surface functions.

Differences in gene frequencies between populations may not only influence HLA and disease associations but also account for differences in disease incidence in populations of different countries, or even between different areas of the same country. Differences in HLA and disease association may also occur as the result of the degree of 'linkage disequilibrium', that is the association of alleles at different loci. It is important to know the incidence of HLA types in the normal population of the area of study. B8 is associated with autoaggressive disorders in white Caucasians but the Japanese equivalent is either B5 or BW35, which are relatively common in this race, whereas B8 is rare. The Japanese also lack DR3 and DR7. In studies of psoriasis (White, Da Costa & Morton, 1972) and of pemphigus (Krain *et al.*, 1973) the fact that A1 is low and A9 high in Jews had to be taken into account.

Of the skin diseases, psoriasis was the earliest to be studied. In 1972 Russell, Schultes and Kuban, and White *et al.* reported a three- to fourfold increased incidence of B13 and B17. B17 seemed to be associated with early onset and this was confirmed by Krulig *et al.* (1975). It may be interesting to recall that Burch and I much earlier in 1965, as a result of analysis of age- and sex-specific incidence rates, predicted that psoriasis was not a homogeneous disease and that there were two genetically distinct subpopulations predisposed to psoriasis and that these were related to early and late onset (Burch & Rowell, 1965). More recently, a study by Brandrup *et al.* (1982) of thirty-two Danish monozygotic twins over the age of 50 years in whom one or both of each pair had psoriasis (eighteen concordant, fourteen discordant) provided evidence that genetic factors are important in the manifestation, age at onset, clinical type, course and severity of psoriasis. A close association of B13 and B17 was present in both discordant and concordant pairs, although psoriasis could develop when neither of these antigens was present. It is now recognized that psoriasis is associated with B37, CW6, DR6 and DR7 as well as with B13 and B17. The primary association appears to be with CW6, although some authors (Tiwari *et al.*, 1982) suggest that DR7 may be the primary association. Alternatively there may be two psoriasis susceptibility genes—one in the DR region (DR7) and the other in the C region (CW6). A family study by Marcusson *et al.* (1978) showed that some patients do not have B13, B17, BW37, CW6 and D antigens associated with psoriasis and suggest that if the predisposing genes are present on HLA they must be in linkage disequilibrium with these genes. Non-HLA factors are known to occur in psoriasis and Beckman *et al.* (1977) found an increased incidence of blood groups (Fya + bb –) and a decrease in haptoglobin type 2-2.

That different types of psoriasis may have a different incidence of HLA types is shown by the finding of Tiilikainen *et al.* (1980), in Finland, that the incidence of CW6 in guttate psoriasis is higher than in ordinary plaque psoriasis. B27 is raised in pustular psoriasis either of the localized, acrodermatitis continua or generalized types, and these are the types of psoriasis particularly associated with a high incidence of seronegative arthritis. There is no increase in B13, B17 or BW37 in pustular psoriasis and this suggests that ordinary psoriasis and pustular psoriasis are genetically different disorders (Zachariae *et al.*, 1977). B13, B17 and B37 are normal in so-called napkin psoriasis, suggesting that this is not true psoriasis. There is an increased frequency of BW38 and DR4 in psoriatic arthropathy but not in psoriasis without arthropathy (Murray *et al.*, 1980). However, there is still some conflict, as Armstrong, Panayi & Welsh (1983) found that DR7 is an additional marker for psoriatic arthropathy. These discrepancies may be explained by case selection as distinct groups of patients with psoriatic arthropathy are related to increased frequencies of certain HLA types (Gerber *et al.*, 1982). Severe arthritics have an increased incidence of DR4 compared with milder cases. Those with mild arthritis have increased A3, B7 frequency which does not occur with moderate or severe arthritis. Patients with rheumatoid-like changes have increased DR4 and B40, and those with spondylitis B27 and CW1. HLA typing may also be useful in therapy. A1 and B8 are more frequent in patients with methotrexate-induced liver cirrhosis as compared with non-cirrhotic psoriatic patients treated with methotrexate (Zachariae *et al.*, 1980). This suggests that the presence of A1 and B8 in a patient should be included in the relative contraindications to methotrexate therapy.

Other disorders which used to be thought to be single entities are now known to consist of two or more subsets. In 1968 Burch and I studied the age- and sex-specific onset rates in chronic discoid lupus erythematosus in series of patients from England, Denmark, Sweden and the United States and predicted that not only was there a genetic predisposition, but also that there were at least three separate genotypes related to the age at onset. HLA typing has tended to confirm this. In our population the genotype with onset between 15 and 39 years is associated with an increased incidence of B7 and the late onset genotype with an increase in B8 in females (Millard *et al.*, 1977). We did not have enough patients with onset before 15 years to confirm any alteration in the expected incidence in the early onset genotype. Tongio *et al.* (1982) could not confirm these findings in French patients but they agree that the predisposing gene in patients under 40 years could be near locus A and in older patients be nearer locus B, giving support to our concept that there may be several genotypes related to age at onset. It is thus important to analyse the incidence of HLA types in relation to age at onset and sex in future studies of all diseases. Subsets defined clinically, pathologically or immunologically may be associated with a particular HLA type. For example, subacute cutaneous lupus erythematosus (Sontheimer, Thomas & Gilliam, 1979), a recently described subset of lupus erythematosus in which photosensitivity, non-scarring annular and psoriasiform cutaneous changes and a high incidence of the cytoplasmic antinuclear antibody anti-Ro, is associated with HLA-B8 DR3 (Sontheimer, Thomas & Gilliam, 1981). HLA-DR3 is particularly frequent in patients with annular lesions and is one of the strongest HLA-disease associations reported.

In systemic lupus erythematosus in Caucasians the most consistent associations are with A1, B5, B7, B8, DR2 and DR3 (Walport, Black & Batchelor, 1982), the strongest association being with B8 and DR3. The relationship between discoid and systemic lupus erythematosus is of considerable interest. The risk of a patient with discoid lupus erythematosus developing systemic lupus erythematosus is about 6% (Rowell, 1984) and about 15% of patients with systemic lupus erythematosus have discoid-like skin lesions. In our experience, the presence of B8 in a female with onset of discoid lupus erythematosus between 15 and 39 years indicates an increased risk of developing the systemic disease (Millard *et al.*, 1977). I believe that discoid and systemic lupus erythematosus are variants of the same disease but are genetically different (Rowell, 1984). Patients who convert must have the predisposing genes for both disorders. Moreover, within each disorder there are several clinical and immunological subsets.

Idiopathic systemic lupus erythematosus can be precipitated in predisposed persons by drugs, but there is a very high incidence of a systemic lupus erythematosus-like illness in patients treated with certain drugs such as procainamide or hydrallazine. The illness usually remits when the drug is withdrawn and the incidence is much higher than the probable gene frequency for idiopathic lupus erythematosus in the population. This suggests that these two entities are not identical. In hydrallazine-induced systemic lupus the important factors seem to be the daily and total doses of the drug, and the acetylator and HLA-DR phenotypes of the patient. There is an increased incidence of DR4 in drug-induced systemic lupus erythematosus as opposed to an increased frequency of DR2 and DR3 in idiopathic systemic lupus erythematosus (Batchelor *et al.*, 1980). The ratio of females to males affected is about 4:1, unlike idiopathic systemic lupus erythematosus in which the ratio is 8:1. When a group of slow acetylators who were DR4 were given hydrallazine all the females developed hydrallazine-induced systemic lupus erythematosus, whereas the only men to do so were receiving 200 mg hydrallazine a day. In practice the determination of acetylator phenotype and DR typing enable susceptible patients to be identified.

An LE-like syndrome occurs in association with inherited deficiency of individual components of complement—C1q, C1r, C1s, C2, C4, C5, C6, C7, C8 or C9. These are rare disorders, the most common being homozygous C2 deficiency; systemic lupus erythematosus occurs in about a third of patients with this deficiency. About 2% of patients with hereditary angio-oedema due to absent or non-functional C1 esterase inhibitor develop systemic lupus erythematosus (Donaldson, Hess & McAdam, 1977). Recently Fielder *et al.* (1983) have found a highly significant increase in the prevalence of null complement alleles in patients with systemic lupus erythematosus, mainly at C4A or B locus, suggesting that null C4 alleles may be risk factors for the disease. The mechanism whereby complement deficiency predisposes to systemic lupus erythematosus is unknown, but it could allow the persistence of virus or other infections or of immune complexes.

In systemic sclerosis some studies have failed to show a clear-cut association with any genetic factor linked to the major histocompatibility complex (Whiteside *et al.*, 1983). However, in our investigations at Leeds (Hughes *et al.*, 1978) we divided our patients into mild and severe groups and found that B8 was associated with the severe cases with visceral involvement and impairment of cellular immunity. Using DR typing Black *et al.* (1984) have shown that DR1, DR3 and DR5 are raised and DR2 decreased in systemic sclerosis. The increase in the frequency of DR3 is attributed to an increase in the A1, B8, DR3 haplotype and suggests that the haplotype pair B8, DR3 is disease related. Patients with mild involvement (so-called CREST syndrome) have raised DR1 and DR5 and decreased DR2 compared with patients with more extensive skin involvement. These authors could find no relationship between any HLA antigen and specific organ involvement, but I suspect that with larger numbers of patients (which could only be obtained from a national survey) and even more sophisticated genetic typing it may be possible to show a relationship between genetic factors and specific organ involvement. Patients with anti-centromere antibodies (a type particularly associated with CREST syndrome) were mainly DR1 or DR5. There was no relationship between HLA type and Scl 70 antibodies, also found in systemic sclerosis. Differences in HLA types reported from different countries are probably due to ethnic differences and gene frequencies in the country. It has not yet been possible to show any association between HLA type and morphoea (Freudenberg *et al.*, 1978). In lichen sclerosus an increase in B49 in females has been reported (Harrington & Gelsthorpe, 1981) but this has not been confirmed (Meyrick Thomas *et al.*, 1982).

An occupational disease with features resembling systemic sclerosis occurs in about one-third of workers exposed to vinyl chloride. There is an increased incidence of DR5 in all patients with vinyl chloride disease and of DR3 and B8 in severely affected patients with features similar to those found in systemic sclerosis (Black *et al.*, 1983) but DR5 may be the predisposing factor as it is found in the whole group. As DR3 and B8 are not found in the milder cases, it has been suggested that they are not predisposing factors but related to the progression of the disease. This argument could also apply to other autoaggressive diseases associated with B8 and DR3.

Another interesting new disease has recently occurred in epidemic fashion in Spain and has been attributed to the ingestion of denatured rapeseed oil. It is a multisystem disorder (Toxic Epidemic Syndrome Study Group, 1982), the late phase of which is characterized by sclerodermatous changes, pulmonary arterial hypertension and a neuromuscular syndrome. It occurs predominantly in females in whom it is particularly severe. This suggests that genetic factors may be important in aetiology which is confirmed by the finding of an increase in DR3 and DR4 in female patients (Vicario et al., 1982).

Among other connective tissue diseases, B8 is increased in juvenile dermatomyositis (Pachman & Cooke, 1980) and more recently it has been found that DR3 is also increased (Friedman *et al.*, 1983).

An increased frequency of DR2 has been found in Wegener's granulomatosis but not in polyarteritis nodosa or Churg–Strauss vasculitis and this is further evidence that these disorders are separate entities (Elkon *et al.*, 1983). Although an increase of B8 has been reported in giant cell arteritis (Hazleman, Goldstone & Voak, 1977) this has not been confirmed (Kemp *et al.*, 1980).

Genetic subsetting is also found in Sjogren's syndrome. The primary disease of dry eyes and dry mouth due to deficient secretion of the lachrymal and salivary glands is associated with an increase in DR2 and DR3 (Manthorpe *et al.*, 1981), whereas in the secondary disease which occurs with rheumatoid arthritis, systemic lupus erythematosus and systemic sclerosis, DR4 is raised. DR4 is also raised in patients with primary Sjogren's syndrome and Raynaud's phenomenon (Mann & Moutsopoulos, 1983). In primary Sjogren's syndrome antibodies to SS-B are often present and these occur more frequently with DR2 and DR3 (Manthorpe *et al.*, 1982). This suggests that antigens or specific immune response genes close to the D region may be related to the production of antibodies to SS-B.

Pemphigus is a good example of a disease in which HLA associations vary with ethnic groups, and control populations for comparison have to be from the same population. The disease occurs particularly in Jews; early studies showed a relation with A10 but a much stronger relationship has now been found with DR4 in Jewish patients (Park *et al.*, 1979). However, even Jews differ. Ashkenazi Jews have increased A26 (a subgroup of A10) and BW38, whereas non-Ashkenazi Jews only have increased A26, suggesting that the primary relationship is with A26 (David *et al.*, 1981). A10 is raised in Japanese patients (Hashimoto *et al.*, 1977) and BW22 in Greeks (Zervas *et al.*, 1979).

Although no one would use HLA typing for specific diagnostic purposes, it may be helpful in distinguishing differences between diseases. Unlike pemphigus, HLA typing is normal in pemphigoid (Ahmed *et al.*, 1977), except in cicatricial ocular pemphigoid in which there is an increased incidence of B12 and possibly A3 (Mondino, Brown & Robin, 1978). HLA-B8 occurs in about 85% of patients with dermatitis herpetiformis (Katz *et al.*, 1977; Buckley *et al.*, 1983) but DR3 is probably the antigen primarily associated with the disease (Pehamberger, Holubar & Mayr, 1981). Initially a higher incidence of B8 was found in patients with associated gastrointestinal abnormalities. Later studies (Seah *et al.*, 1976) found no differences in HLA types between patients with and without jejunal changes. This suggested that dermatitis herpetiformis is a homogeneous disease and is not divided into two groups, in one of which a gluten-sensitive enteropathy is associated with B8. However, dermatitis herpetiformis can be separated on immunohistological grounds into the common form with granular IgA deposits in the dermal papillae and the rarer group with linear deposits of IgA at the dermo-epidermal junction. Patients with the latter rarely have jejunal abnormalities and the incidence of B8 is normal (30%) compared with the former, most of whom have jejunal abnormalities, and the incidence of B8 is 88% (Lawley *et al.*, 1980). This is evidence that linear IgA disease is a different entity from that with granular IgA deposits in the skin.

Several other common skin diseases have also been investigated. The evidence for a connection between HLA and constitutional eczema is contradictory. Raised A3 and A9 (Krain & Terasaki, 1973) and BW35 (Goudemand, Deffrenne & Desmons, 1976) have been reported but Scholz *et al.* (1976) found no alteration. However, this field could be explored further, especially with regard to subsets. Although constitutional differences between individuals might be expected in contact dermatitis, there is no alteration in HLA types in chrome dermatitis in bricklayers (Roupe, Rydberg & Swarbeck, 1979), in patients sensitized to chrome, nickel, formalin, Balsam of Peru (Liden *et al.*, 1981) or para compounds (Valsecchi *et al.*, 1982).

Although it is likely that there is a genetic predisposition to lichen planus, there is no evidence of any HLA association (Veien *et al.*, 1979; Potts & Rowell, 1981), although early reports suggested an association with A3 and B5 (Lowe *et al.*, 1976) and B7 in familial lichen planus (Copeman *et al.*, 1978) and A28 in non-diabetic patients with lichen planus (Halevy *et al.*, 1979). There is no relation between lichen planus and lupus erythematosus (Potts and Rowell, 1981) although some patients show clinical, histological and immunohistological overlap. Such patients probably have both diseases. The position regarding vitiligo is unresolved. Although Retornaz *et al.* (1976) found no overall alteration in HLA frequency, there was an excess of B13 in patients with anti-thyroid antibodies, and Metzker *et al.* (1980) found differences related to age at onset, so there may be several subsets in vitiligo. Recently an association with DR4 has been demonstrated (Foley *et al.*, 1983). The position is also confused in alopecia areata. Alopecia totalis is not associated with any change (Van Neste *et al.*, 1980), whereas B12 is raised in the localized disorder (Kianto *et al.*, 1977), although this is denied (Kuntz *et al.*, 1977). HLA-A29 is raised in granuloma annulare (Middleton & Allen, 1984).

The role of genetic factors in immune deficiency and the prognosis of infection has been under-emphasized. HLA genes may be in linkage disequilibrium with 'susceptibility' or 'resistance' genes at linked loci. Alternatively the HLA genes may affect susceptibility to, or severity of, disease (Mittal, 1977). No alteration in the frequency of A and B antigens has been found in patients with warts (Friedman-Birnbaum *et al.*, 1982) and an increase in A11 in non-atopic patients with scabies (Falk & Thorsby, 1981) needs to be confirmed. That there may be an association of viral disease with an HLA antigen is shown by the increased incidence of A1 in patients with recurrent herpes simplex (Russell & Schlaut, 1975) and CW2 is increased and BW16 decreased in herpes labialis (Blackwelder *et al.*, 1982). It is interesting that an increase in HLA B15 has been reported in erythema multiforme and in particular in those patients with erythema multiforme following herpes simplex infections (Duvic *et al.*, 1983).

There is an increased incidence of the HLA DR5 allotype in male homosexuals with Kaposi's sarcoma as part of the acquired immune deficiency syndrome (AIDS). However, AIDS may also manifest as a disorder with lymphadenopathy, fever and weight loss without signs of malignancy or opportunist infection, and in this condition the incidence of DR5 is also increased (Enlow *et al.*, 1983). Some of these patients later develop Kaposi's sarcoma, and the evidence suggests that the genetic predisposition is associated with the immune deficiency rather than predisposing to the malignancy itself. Thus AIDS can be regarded as a genetically influenced premalignancy. Other malignancies have also been studied. In mycosis fungoides there is increase in AW19 (Dick & MacKie, 1977). There is no alteration in HLA incidence in xeroderma pigmentosum (Giraldo *et al.*, 1977).

Behçet's disease illustrates that a study of antigen splits in disease may reveal new associations, or strengthen presently recognized associations. Early observations from Japan showed a strong association with B5 (Ohno *et al.*, 1973). More recently it was found that in Turkish and British patients the disorder was specifically associated with the BW51 split (Yazici *et al.*, 1980). There are apparently very few diseases associated with antigen splits. Recurrent oral ulceration is a common problem and often is difficult to distinguish from Behçet's disease. Challacombe *et al.* (1976) found an increased incidence in B12 in such patients.

What then is the importance of HLA typing to dermatologists? Firstly it may confirm that there is a genetic component in the aetiology of a disease. Genetic predisposition as a concept is now accepted more freely than 20 years ago, but the new evidence about the toxic oil syndrome, vinyl chloride disease and acquired immunodeficiency syndrome is exciting and indicates that many diseases have a multifactorial aetiology and probably require a genetic predisposition. It helps to determine the relationship between diseases such as psoriasis and ankylosing spondylitis. It helps to define subsets. It is now recognized that many diseases previously thought to be homogeneous really consist of subsets which may be defined clinically by age at onset, sex or by clinical features, organ involvement or severity, as in psoriasis, lupus erythematosus or systemic sclerosis, or immunologically such as the relationship with anti-centromere antibody in systemic sclerosis or IgA deposits in the skin in dermatitis herpetiformis and linear IgA disease.

It is of less use in diagnosis. Despite the strong association with B27, ankylosing spondylitis can be diagnosed on other grounds. On the other hand, there is no specific test for Behçet's disease and demonstrating B5 or better BW51, its antigen split, in a patient may be helpful. Possibly HLA typing might also distinguish herpes gestationis from a toxic eruption of pregnancy (Holmes *et al.*, 1982). HLA typing may have a role in the prognosis of certain conditions. The presence of B8 in a female patient with discoid lupus erythematosus implies an increased risk of developing systemic lupus erythematosus, and B8 in a patient with systemic sclerosis also implies more severe disease and a poorer prognosis.

Clinicians must recognize the pitfalls. Controls for comparison must come from the local population and the numbers must be adequate for correct statistical analysis. It is important to analyse data in several ways, e.g. by age at onset, by sex, by clinical or laboratory features, because subsets may show HLA associations when the total series does not. It is important to remember that the absence of an HLA association does not imply that there is no genetic component in the aetiology of a disease.

What about the future? I feel we have only just disturbed the surface of this field. More emphasis should be placed upon the genetic factors underlying defence mechanisms. The outcome of a disease depends on a balance of attack versus defence, and as the latter is undoubtedly under genetic control this aspect should be studied intensively. Scientists tend to reject negative findings in their research: perhaps in these lies the clue to survival. An individual may not benefit, practically or emotionally, from a print-out showing his or her genetic predispositions, but genetic engineering is developing fast and it may be possible, in the not too distant future, to manipulate genes for the benefit of mankind.

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Comment

AUTOIMMUNE INJURY OF SQUAMOUS EPITHELIUM BY PEMPHIGUS AUTOANTIBODIES

The term pemphigus refers to a group of bullous diseases that are characterized by the presence of both intraepidermal blister formation and autoantibodies specific for epithelial cells (Lever, 1965). Pemphigus vulgaris, the commonest form, is a potentially lethal disease in which the blister occurs just above the basal cell layer. Pemphigus vegetans is an uncommon variant in which vegetating lesions are predominantly localized to flexural areas. Pemphigus foliaceus is a less serious disorder in which the blister is very superficial, occurring just below the stratum corneum. Brazilian pemphigus foliaceus (Fogo Selvagem) is a form of pemphigus foliaceus that occurs in an endemic fashion in Brazil. Pemphigus erythematosus (the Senear–Usher syndrome) is a clinical syndrome in which pemphigus foliaceus occurs in a patient with the clinical and serological features of lupus erythematosus (Senear & Usher, 1926).

All forms of pemphigus are characterized by epidermal cell–cell detachment (acantholysis) leading to intraepidermal vesicle formation, and the presence of IgG antibodies directed against antigenic determinants present on the cell surfaces of differentiating keratinocytes (Beutner & Jordon, 1964). The underlying mechanisms which trigger this autoantibody formation are essentially unknown. The key problem that needs to be resolved is whether there is a breakdown in the self-tolerance of an unaltered antigen (the pemphigus antigen), or whether there is a change in the antigenicity of the antigen itself.

There do appear to be genetic factors which predispose to the development of the disease. Although pemphigus vulgaris is found in all ethnic and racial groups, it has a higher incidence in Jews, and is associated with an increased prevalence of the HLA-DRw4 phenotype (Brautbar *et al.*, 1980; Park, Terasaki & Ahmed, 1979).

Pemphigus has been associated with autoimmune disorders such as lupus erythematosus, rheumatoid arthritis, myasthenia gravis, pernicious anaemia, Hashimoto's thyroiditis and with benign thymomas (Diaz, Glamb & Silva, 1980; Maize, Green & Provost, 1982). Usually the course of pemphigus is not affected by the presence of these disorders. Pemphigus has also been associated with various malignancies, but the incidence of malignancy is not high, and temporal relationships are rare.

Recently, penicillamine has been implicated as a potential causative factor in the induction of pemphigus foliaceus in some patients (Degos *et al.*, 1969; Tan & Rowell, 1976). It has been estimated that pemphigus foliaceus will occur in about 7% of patients receiving long-term penicillamine therapy (Marsden *et al.*, 1976), which makes the association seem more than fortuitous.

Pathogenic mechanisms in pemphigus

Pemphigus vulgaris is a model for organ-specific autoimmune disease. It is a potentially lethal bullous dermatosis characterized by a unique autoantibody (pemphigus autoantibody), specific for an epidermal cell surface antigen normally present in the differentiating keratinocytes of squamous epithelia. Normal individuals are tolerant to this epidermal antigen. Loss of tolerance and autoantibody formation initiates pemphigus.

The autoantibodies in pemphigus vulgaris which can be detected both bound, in and around lesions, and circulating in the serum have not only proved to be crucial for the accurate diagnosis of these conditions, but appear to be of primary importance in the pathogenesis of the diseases as well.

Pathogenic role of pemphigus autoantibodies

Clinical observations. Several clinical observations support the pathogenic role of an autoantibody in the formation of the blisters of pemphigus. By the use of immunofluorescence (IF) techniques, there can be demonstrated in virtually 100% of cases the presence of an IgG class autoantibody bound to the surface of the epidermal cells both in and around lesions (Beutner *et al.*, 1965). Circulating anti-epithelial autoantibodies can be found in the serum in the majority of patients with pemphigus as well, and these circulating autoantibodies are also capable of binding to the epidermal cell surface of normal human skin (Beutner & Jordon, 1964). The antigen to which they bind is not restricted to human skin alone, for these autoantibodies will bind to the cell surface of stratified squamous epithelium from all mammals and birds (Diaz, Weiss & Calvanico, 1979). By using serial dilutions of patient's serum, and applying these to frozen sections of squamous epithelium from an animal source, it is possible to obtain an indirect IF titre for these autoantibodies which is semiquantitative. It has been observed in the majority, but certainly not in all patients with pemphigus, that this indirect IF titre for pemphigus autoantibodies correlates well with the extent of disease present in the patient (Castro, Roscoe & Sampaio, 1983; Fitzpatrick & Newcomer, 1980; Sams & Jordon, 1971).

Plasmapheresis has been employed for the treatment of patients with pemphigus (Auberbach & Bystryn, 1979; Roujeau *et al.*, 1983; Swanson & Dahl, 1981). The rationale for this treatment is that removal of circulating autoantibodies, with subsequent decrease in the IF titre of these autoantibodies, will lead to clinical improvement in the disease. Intensive plasmapheresis does produce a decrease in circulating autoantibody titres and an improvement in the disease activity of the patient, but the improvement is very short-lived. With the cessation of plasmapheresis the autoantibody titres quickly rebound, sometimes to a higher level, and the patient's disease flares once again (Roujeau *et al.*, 1983).

There are several documented cases in which a mother with pemphigus vulgaris has given birth to a child whilst her disease was active, and this child was born with lesions typical of pemphigus vulgaris (Moncada *et al.*, 1980; Storer *et al.*, 1982). These infants were also shown to have IgG antibodies bound to the epidermal cell surface in and around lesions. Without treatment, the disease improves spontaneously after birth, suggesting that maternal IgG was responsible for induction of the disease.

In vitro studies. Many *in vitro* studies strongly support the pathogenic role of pemphigus autoantibodies in the induction of acantholysis. Bellone and Leone (1956), Schiltz and Michel (1976), Morioka, Naito & Ogawa (1981) have demonstrated that pemphigus serum or the IgG fractions from pemphigus serum induce acantholysis in human skin explant cultures. Farb, Dykes and Lazarus (1978) and Diaz and Marcelo (1978) have shown that pemphigus serum when added to murine primary epidermal cell cultures will cause epidermal cell detachment. This cell detachment is highly reproducible and quite specific for pemphigus autoantibodies. Our laboratory has shown that such cell detachment does not occur when cultures are treated with normal human IgG or IgG fractions isolated from the serum of patients with bullous pemphigoid or lupus erythematosus (Woo *et al.*, 1983). This is also not reproduced by the use of

IgG specific for blood group substances A and B which are expressed on the epidermal cell surface. These cell culture systems are now being employed by several groups of investigators to try to determine the sequence of events that occurs subsequent to autoantibody binding with the epidermal cell surface. This is done in an attempt to determine the molecular mechanisms by which this very unique form of cell detachment occurs. At present, little is known about the mechanisms by which this phenomenon occurs, but there is increasing evidence that the activation or release of proteolytic enzymes by epidermal cells may be a key event.

Animal models for pemphigus. We have shown that, given in sufficient doses, IgG fractions purified from the serum of patients with pemphigus induce a disease in neonatal BALB/c mice which reproduces the clinical, histological, and immunological features of the human disease (Anhalt *et al.*, 1982) (Fig. 1). The neonatal mouse was chosen as a model for several reasons. Firstly, primary epidermal cell cultures derived from neonatal BALB/c mice have proved to be a very reliable system for testing various effects of the autoantibodies *in vitro* (Woo *et al.*, 1982, 1983). Secondly, neonatal mice have been shown to be immunologically tolerant to injected foreign serum proteins (Bach, 1978). This eliminates the concern that the animal may mount an antibody response against injected human immunoglobulins, thereby altering the subsequent reaction. Thirdly, because of their very small size, relatively large amounts of human IgG can be administered to these animals. In the study we reported, we used IgG doses from 1·5 to as high as 16·0 mg per gram body weight of mouse per day. These injections were intraperitoneal and

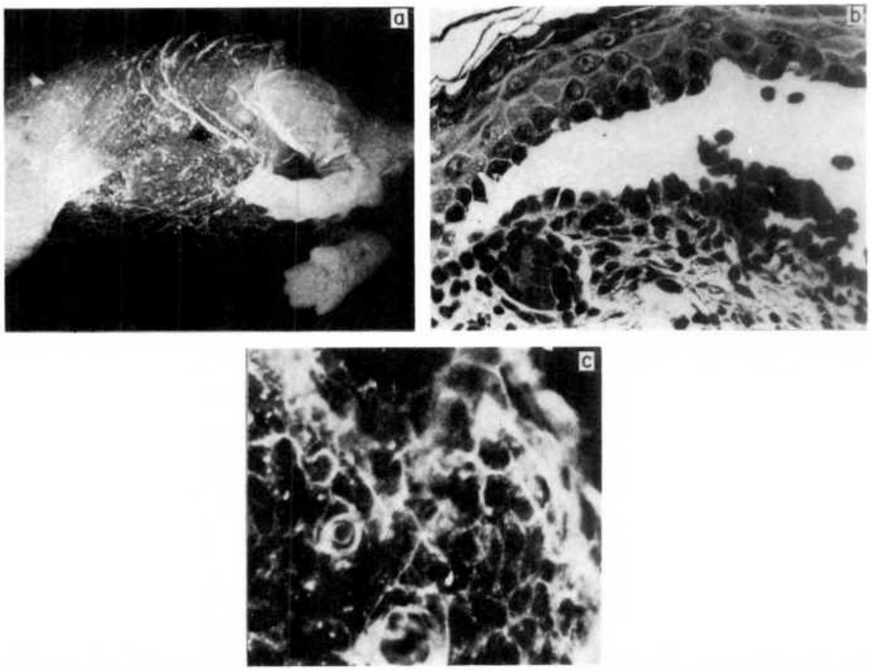

FIGURE 1. Neonatal BALB/c mouse injected intraperitoneally with human pemphigus vulgaris IgG. (a) Epidermal injury occurring 48 hours after the initial dose of IgG manifested clinically by a Nikolsky positive dermatosis. (b) The epidermal vesicles are intraepidermal and show acantholysis (H & E, × 400). (c) Direct immunofluorescence studies of the skin of these animals shows binding of pemphigus IgG to the epidermal intercellular spaces (× 400).

given in two divided doses daily. Within 18 to 72 hours of the beginning of injections, mice injected with pemphigus immunoglobulins developed cutaneous blisters and erosions. The key aspects of the investigation show that the lesions produced by passive transfer of human pemphigus immunoglobulins simulated the lesions seen in the human patients clinically, histologically, ultrastructurally and immunologically. We found that the extent of disease that was produced in these animals was dependent both on the total dose of IgG administered, and the pemphigus autoantibody titre of these IgG fractions (as measured by indirect IF). These cutaneous lesions were not observed in any animals that received comparable doses of pooled normal human IgG, and in those animals receiving pemphigus immunoglobulins, lesions would occur only as long as injections were continued. Once the injections were discontinued, new lesions would cease to appear and previous lesions would begin to heal and resolve.

IgG fractions obtained from the serum of patients with extensive cutaneous disease and high titres of pemphigus autoantibodies produced disease in these mice reproducibly. IgG fractions from those patients who had limited disease and low titres of pemphigus autoantibodies did not produce significant disease in the mice, when given in comparable doses. We also had the opportunity to obtain large volumes of plasma from a single patient with untreated pemphigus both prior to treatment, during his courses of therapy (with prednisone and cyclophosphamide) and when his disease was in clinical remission. The indirect IF titre for pemphigus autoantibodies was initially very high (1:400) and fell to 1:100 during the early convalescent phase. IgG fractions from his pretreatment serum were most effective in inducing cutaneous lesions in our model, and the IgG from the convalescent phase was ineffective. The IgG fractions from serum obtained during treatment were intermediate in potency.

In summary, the evidence that pemphigus autoantibodies are of primary pathogenic importance in the disease is as follows:

(a) IgG class autoantibodies can be found both circulating in the serum of patients with pemphigus vulgaris and bound to the epithelial cell surfaces in and around lesions.

(b) These autoantibodies purified from the serum of pemphigus patients, can induce acantholytic lesions typical of pemphigus both in experimental animals (neonatal mice) and in human and murine epidermal cell cultures.

(c) These autoantibodies react with a specific antigen of the epidermal cell. This purified antigen has been used to immunize rabbits and the resulting antibodies are capable of inducing pemphigus-like lesions in neonatal mice (Peterson & Wuepper, 1983).

Witebsky, Rose and Terplan (1957) put forward several criteria that ideally should be fulfilled in order to prove the pathogenic role of an autoantibody in a putative autoimmune disease. Those criteria have been fulfilled for pemphigus vulgaris. There can now be very little doubt of the autoimmune nature of this disease and of the primary role of autoantibodies in this disorder.

Mechanisms of epidermal cell injury by pemphigus autoantibodies

Schiltz, Michel & Papay (1979) found that the supernatants of cultures treated with human pemphigus IgG contained a proteolytic enzyme that could then cause acantholysis when added to fresh skin explants. It has also been shown that the increase in cell detachment that can be observed in epidermal cultures treated with pemphigus IgG can be abolished by certain proteinase inhibitors (Woo *et al.*, 1983). This would suggest that pemphigus autoantibodies may cause the activation or release of certain proteolytic enzymes following their binding to the epidermal cell surface. These released enzymes may be responsible for this cell detachment. Recently, Hashimoto *et al.* (1983) identified a plasminogen activator (PA) in the supernatant of human epidermal cell cultures that had been treated with pemphigus IgG. Their hypothesis is that the increase in PA, with subsequent activation of plasmin, may be responsible for the cell–cell detachment. This is strengthened by the fact that treatment of these cell cultures with dexamethasone will abolish PA activity in the cell cultures. This would presumably explain why corticosteroids are clinically beneficial. Unfortunately, they did not show that the incorporation of dexamethasone produced any net change in cell detachment or acantholysis, thus failing to prove whether this increase in PA activity was secondary to cell detachment, or was of primary importance.

We have made some observations that would seem to indicate that this system may not be of primary importance in the induction of acantholysis. In murine epidermal cell cultures treated with pemphigus IgG, we have demonstrated that the incorporation of dexamethasone can abolish PA activity almost completely in these cultures, but quantitative cell detachment remains unaffected by treatment with the drug (Woo *et al.*, 1982). Furthermore, in our *in vivo* mouse model of pemphigus, we have shown that treatment with even suprapharmacological doses of dexamethasone does not affect the extent of disease induced by passively transferred pemphigus IgG (Anhalt *et al.*, 1983). Control animals, receiving identical doses of pemphigus IgG but no dexamethasone develop similar disease activity at the same time points. This would indicate that corticosteroids produce their major therapeutic effect *in vivo* by reducing autoantibody production, rather than by the other effects they exert at the target tissue level.

In a recent study Takahashi *et al.* (1984) have shown that the ultrastructural changes detected in the epidermis in the murine model of pemphigus, which include widening of the intercellular spaces, separation of the desmosomes into split desmosomes, dissolution and disappearance of attachment plaques, and perinuclear tonofilament retraction, are all consistent with previously reported findings in pemphigus in humans (Hashimoto & Lever, 1967) and in the experimentally induced acantholysis in human skin explants (Hu, Michel & Schiltz, 1978). What is most interesting is that the fate of split desmosomes (attachment plaques and tonofilaments) in pemphigus antibody-induced acantholysis is unique and quite unlike that observed in epidermal cells that are detached from each other by proteases such as trypsin. It has been known for years that in trypsin-dissociated cells the split desmosomes are internalized into the cytoplasm, but the attachment plaques and tonofilaments remain intact for a period of time, and if the cells reaggregate, these split desmosomes can reform intact desmosomes between adjacent cells (Overton, 1968). The difference in the behaviour of the attachment plaques and tonofilaments in the detachment of epidermal cells that is induced by pemphigus antibodies, as opposed to that observed with trypsin dissociation, would suggest that acantholysis in the human disease may not be due solely to extracellular protease activity. The importance of intracellular events in the rounding up and detachment of the cell remains to be clarified.

Some initial observations on such intracellular events have been reported by Patel *et al.* (1984). They studied the fate of the pemphigus antibodies after they bind with the cell surface of murine keratinocytes *in vitro*. By immunoelectron microscopy, they showed that there is a dynamic sequence of events that occurs after their binding with the cell membrane. Within 30 minutes, the pemphigus antibodies that were initially evenly distributed on the cell surface had become clustered. Subsequently, these clusters of antibody were internalized into submembranous vesicles via surface pits, and fused with lysosomes within the cell. These changes coincided with the development of widening of the intercellular spaces between adjacent keratinocytes. This internalization of the pemphigus receptor–antibody complex may be very important in the detachment process.

Therefore, it is conceivable that the epidermal acantholysis induced by PV antibodies may go through an early detachment process, perhaps mediated by proteases as suggested by several investigators (Farb *et al.*, 1978; Schiltz *et al.*, 1979; Woo *et al.*, 1982). However, the fully developed acantholytic vesicle may represent the end result of several cytopathological processes that perhaps are operating within the keratinocyte itself rather than in the extracellular milieu alone.

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An appreciation

Marion B. Sulzberger

Born 1895, died November 1983.

I cannot hope to detail all the history of this remarkable man. For several years I had urged him to write an autobiography, and he was engaged on this effort when he passed away. He had to be persuaded, for he had no exaggerated ideas of his achievement.

Early experience of the world explains his tolerance, and the ability to appreciate the qualities of all men, whatever their eminence or social status, which he showed throughout his life. He was a great lover of humanity, and a practical one, who did not love his fellows at a distance or from a theoretical, spiritual or ethical compulsion imposed by religious teachings. His humanity was based on his large heart and his wide experience of human behaviour and his acceptance of human qualities.

His life was a romantic one from boyhood. He left Harvard after a brief stay, as he did not find the establishment 'congenial', and he left home at the age of 17, working as a deck hand, stoker on a merchant ship, and then in Australia as a camel driver and later in an up-country sheep station. There, he was alone, and was visited only once a month with supplies. He was otherwise left to cope with a large number of sheep over an extensive isolated area, where he was dependent on his horse to get him back to his base. After this varied experience he did a survey of cattle lands in Paraguay and Argentina, spent 2 years in business, and in 1917 joined the Navy as a Lieutenant j.g. U.S. Navy. He became a pilot and instructor in the Air Branch.

In 1920, at the age of 25, he went to Geneva to see his father who had gone to Switzerland in the hope of a cure for his fatal illness. While in Geneva, Sulzberger was attracted by the personality of the physician who had attended his father, and there and then he decided to enrol at the university as a medical student. He qualified M.D. at the age of 31 in Zurich, and, in turn, his enthusiasm for medicine was diverted to dermatology by the plight of a young relative who was suffering from severe acne. His career spanned a period of great significance in dermatology.

He first worked with Bruno Bloch who was an eminent scientist and whose publications on the synthesis of melanin were fundamental. It was with Bloch that Sulzberger described incontinentia pigmenti. He subsequently held a post with Joseph Jadassohn in Breslau and also visited Unna and Miescher. Sulzberger told me that Unna, who was not popular in his day, had contributed very significantly to the advancement of dermatopathology, particularly by the use of the then new azo dyes for identification of cell types in sections.

When Sulzberger left for New York in 1929, with his drive, scientific ambition and scholastic grounding, he was more than well fitted for his future task. He became Head of the New York Skin and Cancer Hospital. This hospital, largely self-supporting, had very busy clinics, patients paying a small fee when possible. There was a large unpaid staff and a few full-time scientific workers who had to make do with very limited accommodation and means. However, the output of scientific and clinical papers to which Sulzberger contributed was remarkable. The Postgraduate School was established, and became the largest in the U.S.A., giving a most comprehensive training in dermatology and syphilology. When I visited New York in the early sixties, I envied the postgraduates for the training available. Sulzberger had taken in and had been very generous to many refugee dermatologists from Hitler's Germany, including Jessner, Biberstein and Franz Herrmann, who returned to Germany after the war as professor of dermatology.

When Sulzberger became a public figure with an urge to improve the scientific basis of dermatology, he was met by opposition from many of his contemporaries and seniors, but he persevered in ensuring that basic training was available, and he was one of those instrumental in the establishment of the American Board Examination. Although it was a hard struggle, I have not once heard him denigrate his fellows during my years of enjoying his friendship. He never complained about personal trauma.

From the Postgraduate School, graduates went to all parts of the United States and Latin America. Sulzberger was proud of his graduates, and they, in turn, directed their gratitude and affection to him. He showed undiluted pleasure when he spoke of his postgraduates and their careers. Only recently he told me that four of them occupied posts as heads of University Dermatological Departments in New York alone.

He kept up an extensive correspondence with dermatologists asking for information and help or requesting articles, and, partnered by his wife Roberta, he was tirelessly pursuing the furtherance of the activities of the Institute of Dermatologic Communication and Education which they had created. From the inception of this foundation, it was Roberta who was and still is the administrator, and it was she who was most active in the preparation and distribution of the teaching material. Their teaching films were a feature of international congresses and meetings of the American Academy of Dermatology. They were an extraordinary vehicle for the instruction of the young and not-so-young dermatologists of the Americas and the whole world.

Sulzberger was prominent in the establishment of the prestigious *Journal of Investigative Dermatology* of which he was the first editor from 1938 to 1950. He also established the Year Books of Dermatology, which he edited from 1931 to 1953. He wrote or contributed chapters to 26 textbooks, mainly concerned with dermatological topics. The textbook which he regarded as his best achievement was *Dermatologic Allergy*, published in 1940.

The original articles by Sulzberger, alone or in collaboration, reach the astounding figure of 432. The topics were varied and indicate the range of his investigations and interests. The list includes: allergy as it affects the skin; the actions of war gases, their prevention and treatment; insect repellents; drug hypersensitivity, its prevention and hyposensitization; clinical effects of disturbance of sweat delivery; trichophytides; industrial dermatosis; poison ivy; and the use of topical steroids on the skin under occlusion (with Witten), which they were first to advocate.

He was an early member of the Allergy Club of New York, in the company of such eminent scientists as Coca and Landsteiner, who was later to receive the Nobel prize for Medicine for his discovery of the blood groups, and who was to create the concept of the hapten. They met every Sunday morning, and Sulzberger told me that it was at one of these meetings that the word 'atopy' was coined by Coca after a discussion on the allergic diathesis.

Another of Sulzberger's tales involved Landsteiner; while working in Zurich with Bloch, Sulzberger found that he could induce, in a guinea-pig, tolerance to the application of a sensitizing arsenical compound by preliminary intravenous injection of the same substance. Sulzberger was successful in Zurich, but back in New York the results could not be repeated. Landsteiner was asked to help but could not confirm Sulzberger's previous findings, nor, in fact, did he believe that it was theoretically feasible. However, when the guinea-pig strain which Sulzberger had used in Zurich was brought to New York, the experiment was found reproducible after all. This story was retold by Sulzberger when, some years ago, the medical and scientific world was concerned with claims of experiments which could not be repeated, and where there was a suggestion of deliberate or subconscious deception. Sulzberger stressed that absolutely all the conditions of the original techniques had to be fulfilled before the validity of an experiment could be questioned.

In April 1940, he was made Lieutenant Commander U.S. Naval Reserve, and from 1941 to 1946 he was on active duty and promoted to Captain of the U.S. Naval Reserve Medical Corps and Consultant to the Bureau of Medicine and Surgery, U.S. Naval Department. In 1949 he received the Legion of Merit and Permanent Citation from the Naval Department, and in 1950 he received the New York State Conspicuous Service Cross.

After he retired from New York, Sulzberger spent some years as Senior Scientific and Medical Adviser to the Surgeon General at the Pentagon, where, he said, he worked harder than at any time before in his life. He kept this title even after going to live in San Francisco, where, very typically, he was able to persuade the military authorities to establish a research department at the large Letterman Military Hospital. I had the privilege of visiting this department and saw the graduates working with Sulzberger on relevant problems, such as friction blisters, the induction of miliaria and consequent anhidrosis, and also on insect repellents.

Sulzberger was honoured by dermatological societies worldwide. The list of his honorary memberships of dermatological societies reads like a travelogue. He was honorary and corresponding member of 35 dermatological societies in 28 countries outside the United States, and of 20 American societies. He was made an Honorary Member of the British Association of Dermatologists in 1946, and at the time of his death he was its most senior honorary member. He was an active member and chairman of the International Committee on Dermatology from 1957 to 1972. His stature and negotiating ability contributed much to the cohesion of the committee and to the furtherance of its work. His fluency in German, French and Spanish was a great help at international meetings. His comments on 'My Companions in the International Committee of Dermatology', published in the Journal of the American Academy in 1982, give interesting vignettes on the important figures on the international scene.

He was a strong anglophile and loved coming to Britain. In 1949 he gave the Prosser White Oration at the St John's Hospital Dermatological Society, and in 1952 he was Vice President at the International Congress of Dermatology in London.

The list of professional and other honours bestowed on him includes: Gold Medal of the American Academy of Dermatology; plaque presented by the United States Army Medical Research and Development Command in recognition of distinguished service; the Meritorious Civilian Service Medal, United States Army; and the creation of the Annual Marion B. Sulzberger Prize Lecture by the American Society for Allergy and Immunology in 1979. In 1960 he was awarded the decoration of Chevalier de la Légion d'Honneur by the President of the French Republic.

The dermatologists of Great Britain felt honoured when the Royal Society of Medicine, on the recommendation of the Section of Dermatology, chose to confer the Honorary Fellowship of the Society on Sulzberger in 1982. He was the first overseas dermatologist to be so recognized. In spite of ill health which had pursued him for 6 years, he made the great effort to come to the ceremony at the R.S.M. and was an impressive figure among the distinguished company. The quality and stature of the man was obvious when he spoke. On this visit, he had a full programme of lectures and talks which were greatly enjoyed by the staff of various London hospitals.

His formal lectures given by invitation to the many associations and universities with which he was connected in the U.S.A. and worldwide, were memorable for their clarity and lively delivery. His excellent memory enabled him to draw on his vast experience, and the complete dedication and love which he felt for dermatology were always communicated to his audience.

He was a giant among men, but he was also a wonderful friend and always compassionate and eager to help. Our sympathies go out to his wife Roberta, who was his constant companion and collaborator during their 25 years of marriage. He often stressed the help which she gave him. I have been fortunate to have observed them at work together at San Francisco and at their summer house at Cape Cod. It is a great privilege to have known him.

LOUIS FORMAN

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Correspondence

The association between lichen sclerosus et atrophicus and autoimmune disease in males—an addendum

SIR, Since our recent letter on this subject (*British Journal of Dermatology* 1984, 111, 122) we have seen four further men with histologically confirmed lichen sclerosus et atrophicus, and of these one had alopecia areata. Combined with our previous data, this gives a patient group of twenty-nine, of whom six have an autoimmune-related disease: using Fisher's exact test, the occurrence of an autoimmune-related disease in six out of twenty-nine patients compared with zero out of twenty-five controls is significantly different at the level $P=0{\cdot}037$, which is within the generally accepted confidence limits for statistical significance. We have also studied four more controls, none of whom had an autoimmune-related disease; including these, the control group totals twenty-nine, and the difference between the occurrence of an autoimmune-related disease in the twenty-nine patients compared with the twenty-nine controls is significant at the level $P=0{\cdot}0235$. We recognize that we studied only a small number of patients (as often occurs when making observations on a relatively uncommon disease), but we feel that this data supports our belief that there is an association between lichen sclerosus et atrophicus and autoimmune disease in men.

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Hendersonula toruloidea and *Scytalidium hyalinum* infections in Tobago

SIR, The mould fungus *Hendersonula toruloidea* was first recognized as a human pathogen in 1970 (Gentles & Evans, 1970) when it was found to cause infections similar in clinical appearance to dermatophytosis due to *Trichophyton rubrum*. In 1977 Campbell and Mulder described a similar disease caused by *Scytalidium hyalinum*. *H. toruloidea* is a plant pathogen affecting fruit and nut trees and is found in nature mainly in the tropics and subtropics. *S. hyalinum* has not been isolated from the environment. Infections caused by these organisms have been described predominantly in temperate areas (Hay & Moore, 1984), although the patients affected have usually originated from the tropics. The investigation described here is a preliminary survey carried out on the Island of Tobago, West Indies, to estimate the prevalence of the infection in the general population.

Forty-five patients from the Island of Tobago were surveyed. All were medical in-patients and were receiving treatment for disorders other than skin disease. There were twenty-four females and twenty-one males and their ages ranged from 11 to 82 years. Scrapings taken from soles or toe webs were examined by direct microscopy, and cultured on Sabouraud's agar which did not contain cycloheximide (actidione) which is inhibitory to the growth of Hendersonula and Scytalidium.

Scaling of the sole was noticed in thirty-one patients, toe web cracking or scaling in eighteen patients. Toe nail dystropy compatible with onychomycosis was recorded in twenty-two patients. Otherwise the most common nail abnormalities were splitting of the nail plate and onychogryphosis. All the patients wore shoes or sandals although three occasionally went barefoot. *H. toruloidea* was isolated from three patients (all had positive direct microscopy). *S. hyalinum* was found in seven patients of whom four had positive direct microscopy. The dermatophyte *T. interdigitale* was cultured from one patient. In addition hyphae showing the characteristic features of *H. toruloidea* or *S. hyalinum* (Moore, 1978) were seen in direct microscopy in an additional nine patients but organisms were not isolated.

The survey shows that ten (28%) of the population surveyed in Tobago had an infection caused either by *H. toruloidea* or *S. hyalinum*. In nine of these patients scaling lesions on the sole, typical of the infection, were present. Changes compatible with onychomycosis were seen in eight individuals but nails were not cultured. If those patients with positive direct microscopy of typical appearance but negative cultures are assumed to have a Hendersonula or Scytalidium infection, then it would appear that 45% of the population surveyed are affected. As none of the patients were aware of the problem, it is likely that infections caused by *H. toruloidea* or *S. hyalinum* are a common, but asymptomatic, disease in this area. Similar surveys have not been attempted in other tropical countries but the results may be similar. None of the patients showed palm or finger nail infections which are often the presenting features in a temperate climate and it is possible that spread of the infection to these sites may be affected by climate. As the organisms are inhibited by cycloheximide which is usually incorporated into media used to isolate dermatophytes, the presence of these infections is unlikely to have been detected previously where routine cultural procedures were used.

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Book Reviews

The Cutaneous Arteries of the Human Body. CARL MANCHOT (1983) Berlin: Springer. Pp. 149. Price DM 165.

Carl Manchot was a German Anatomist of the late 19th century who studied the arterial supply to the skin. The work had been largely forgotten until advances in plastic surgery led to the recommendation that Manchot's observations had considerable relevance. His observation that the arteries which supply muscles often supply the overlying skin is especially important. The musculo-cutaneous flap by which skin from far-off sites is transplanted complete with the blood supply of its underlying muscle has been extensively used to improve auto-grafting.

This translation of Manchot's otherwise obscure work, with an introduction by W.D.Morain, is prefaced by a story of the detective work of the author which led to its full documentation complete with biography and the history of the development of this aspect of plastic surgery. It is all nicely done and easy to read but it should be noted that it is concerned with supply to the skin, and documentation of blood supply within the skin is not part of the story.

T.J.RYAN

Cutaneous Toxicity. (Target Organ Toxicology Series). Edited by V.A.DRILL AND P.LAZAR (1983) New York: Raven Press. Pp. 274. Price $60.

This is an updated version of the book with the same title by the same editors, published in 1977. It provides an overview of procedures, with the relevant references rather than technical detail, and usually comment rather than a critical review. The nineteen chapters provide examples of the application of a wide range of techniques to studies on toxicology of skin.

Apart from chapters on the standard procedures to examine potential irritancy of substances, potential to sensitize and potential to penetrate skin, the editors have been more adventurous by introducing chapters on non-invasive techniques for determining skin function (Maibach *et al.*), cutaneous DNA repair mechanisms (Ross & Carter), and an animal skin model for evaluation of topical antimicrobial agents (Sedlock *et al.*). There is, however, a short inadequate chapter on functions of the immune system that does not appear strictly relevant to the title of the book. There is also some lack of perspective, in that in two chapters on photoreactivity, the participation of complement in phototoxicity is given equal prominence with photoallergy. Though undoubtedly both occur, the number of substances shown to induce a phototoxic complement-mediated effect is small compared to the number inducing photo-allergy.

The response of skin to cosmetics, plants and drugs is briefly discussed in separate chapters, and there is an inadequate final chapter on occupational dermatoses that could with advantage have been more detailed, and been used to form a final perspective of the preceding chapters.

W.E.PARISH

Malignant Melanoma; Advances of a Decade. (Pigment Cell Vol. 6). Volume and Series Edited by Rona M. Mackie (1983). Karger.

Melanoma continues to fascinate and frustrate at both a scientific and clinical level. The increasing incidence of cutaneous melanoma is being accompanied by earlier diagnosis in many countries, but cure for disease that has spread remains elusive.

Professor MacKie's book is a concise and easily readable collection of ten essays by authors from Britain, Europe, U.S.A., Australia and Japan. The scene is well set with a masterly distillation of the sometimes contradictory epidemiological findings. While the role of cumulative ultraviolet radiation is generally accepted, it is clearly not as straightforward as with non-melanoma skin cancer, and the importance of other factors including exposure early in life, sunny vacations, sporadic dysplastic naevi and controversial issues such as exposure to certain chemicals and fluorescent tubes are discussed.

With the proviso that prognostication can never be exact for the individual tumour, there follows a review by Schmoeckel of the histological features that can give useful prediction, with the conclusion that thickness and mitotic index are the most useful independent variables. The difficult subject of recognizing the very early melanoma and simulants of melanoma is tackled by Cochran, and he rightly discards the term 'borderline', although is unclear (or just undogmatic?) about whether atypical melanocytic hyperplasia can be said to be melanoma *in situ*. There follows a useful appraisal of the immunological aspects of melanoma, although what to do about an antigenically distinct tumour that usually confounds its initially immunologically competent host remains a central problem. The biochemical markers of melanocyte growth, particularly cysteinyldopa, are discussed by Rorsman and then the evidence for steroid receptors on melanoma cells is discussed, but with no widely applicable conclusions. For the dermatologist the two chapters containing most new information are those on ocular melanoma, where new investigative techniques and conservative management may improve prognosis, and the distinctive acral melanoma as seen in Japan (50% of melanoma in that country). Not all will agree with the surgical recommendations on margins and elective lymph node dissection, but these are still controversial matters. The authors do not discuss the neonatal treatment of bathing-trunk naevus, nor regional perfusion with cytotoxic agents. The closing chapter is on adjuvant chemo- and immunotherapy, at present disappointing.

Criticisms of this timely book are few. It would have been helpful if all the authors had provided conclusions, and perhaps perspectives for the future. In one chapter the references appear as names, in the others as numbers in the text, an editorial slip that is perhaps a measure of the commendable speed with which this book has appeared.

C.KENNEDY

Pocket Atlas of Dermatology. G.K.STEIGLEDER AND H.I.MAIBACH (1984) Stuttgart: Georg Thieme Verlag. Pp. 275. Price £9.85.

Howard Maibach has joined Professor Steigleder to produce this English edition of *Taschenatlas der Dermatologie* which originally appeared in 1981, and forms a companion volume to the pocket textbook, *Dermatologie und Venereologie*, also available in English translation. This edition is almost identical in content to the German one but there have been minor revisions and additions. AIDS, for example, has now been added to the list of sexually transmitted diseases, and a useful list of recommendations for further reading is included as an appendix.

At the present time there are several atlases of dermatology of varying quality on the market, but Professor Steigleder's volume is certainly among the best. It is beautifully produced, comprehensive and truly pocketable. The quality of the illustrations is of very high standard and the balance of text and clinical photographs is just right, making a most attractive work for use on its own, or in conjunction with its companion volume.

NEIL SMITH

Vitiligo and Other Hypomelanoses of Hair and Skin. J-P.ORTONNE, D.B.MOSHER AND T.B.FITZPATRICK (1983) New York: Plenum Medical. Pp. 683, 284 illustrations. Price $79.50.

This is an excellent book on vitiligo and other disorders of pigmentation in which there is a lack of melanin in both the skin and hair. The authors have produced a very comprehensive monograph and one which I enjoyed reading.

The initial chapter on skin colour and the melanin pigmentary system is, as expected, right up to date and provides a good introduction to understanding the pathogenesis of disorders of skin colour. The second chapter on the approach to the problem of leucoderma is of help to the clinician, though perhaps it is rather daunting to see a list of over 90 conditions in which there is hypomelanosis in man, including some such as the Fisch syndrome and kappa-chain deficiency that the reviewer had not heard of before. Later on in the book I discovered that these are very rare and I eagerly await my first case of 'Bird-headed' dwarfism.

A large portion of the book is devoted to the common disorder—vitiligo. The clinical features, associated disorders, pathology, pathogenesis and treatment of vitiligo are all fully covered. At the end of this section there are 743 references, which must be a record. This in itself indicates how thorough the authors have been in researching the literature on such an important topic. Other disorders, such as piebaldism and the various types of oculo-cutaneous albinism are well described. There are chapters on chemical hypomelanosis, hypomelanosis associated with inflammation and also one on those infectious and parasitic disorders that result in hypomelanosis of the skin.

Of interest is the chapter on halo naevus and the leucoderma that is rarely associated with malignant melanoma. This topic fascinates clinicians, immunologists and oncologists. A point that is made is that the halo around benign moles is round or oval and almost always has a regular shape. The mole is right in the centre. In contrast, the halo around a melanoma is irregular and the tumour is eccentrically placed. This point, that is also well illustrated, is an indication of how useful the book is for the clinical dermatologist.

The book is profusely illustrated and there are many excellent black-and-white clinical photographs. All the common and most of the rare disorders are shown, as are their variants. However, there was no picture of a 'Bird-headed' dwarf, but I suppose that this is so distinctive that I should not miss one should he or she turn up one day in my clinic!

The book is reasonably priced and should be bought by all who have an interest in disorders of pigmentation and by most pigment cell biologists. It is essential for every hospital library.

S.S.BLEEHEN

The Dynamic Body Tissues. W.S.BULLOUGH (1983) Lancaster: MTP Press. Pp. 165.

Despite its complexity, dermatologists maintain an active interest in the growth control of their chosen tissues; consequently, it is to be expected that they would turn eagerly to this *envoi* from the *doyen* of the enigmatic chalone hypothesis. There will be the (largest) group, with no specialized knowledge, who will hope for an authoritative, up-to-date statement, accurate and balanced. A further group, themselves experts in the field, might wish for a more critical account of the chalone hypothesis in the context of competing hypotheses. Unfortunately, both of these groups will be disappointed in what amounts to a pro-chalone polemic which is so uncritical as to be acceptable *in toto* by only the most blinkered partisan of the concept.

The chalone hypothesis has been with us for some 25 years or more, and has seen the advent of the G_2 and G_1 chalones, at first regarded as pure cell cycle modulators, but now endowed with powers to control the differentiation rate and the post-mitotic ageing rate of epidermal and other cell types; this complex choreography of cell proliferation and differentiation is controlled by concentration gradients of the inhibitory chalones and a putative 'mesenchymal factor' emanating from the dermis, with all agents operating via specific ligand:receptor binding. It is most unfortunate that insufficient evidence is given to the facts that neither the epidermal chalones nor the mesenchymal factor have been purified or sequenced, chalone receptors on epidermal cells have not been localized or isolated, and there is no experimental evidence for an action of chalones on the differentiation rate. Moreover, scant attention is paid to the solution of those perennial chalone problems, the design of appropriate proliferation assays or the choice of appropriate controls in experiments.

In addition, the chalone concept is advanced to the exclusion, or negation, of any counter-proposal: there is no role for growth factors, notwithstanding recent progress in isolation, sequencing and receptor recognition; perhaps the most powerful model of epidermal organization yet proposed, the ***epidermal proliferative unit*** model, is cursorily dismissed; on a broader front, the human epidermis does not show ordered structure (despite the recent work of MacKenzie); the G_0 phase of proliferative rest is discarded without reference to the convincing multiparameter flow cytometric studies of Darzynkiewicz, and latterly Gelfant on human epidermis; epidermal organization is discussed in detail without even bare consideration of recent concepts of clonogenic and stem cell subpopulations; epidermal differentiation is held to be totally dermis-dependent, without mention of recent observations that epidermal keratocytes stratify and differentiate in serum-free media; the Gompertzian growth of tumours is explained solely by chalone-mediated tumour self-retardation, to the exclusion of P→Q cell transition and cell loss mechanisms; tumour cells retain chalone responsiveness, and grow abnormally only because the local chalone concentration is low, while the alternative hypothesis, supported by experimental evidence, suggesting that chalone responsiveness is lost during neoplastic transformation, is not mentioned; the normal pathway of neutrophil granulocyte destruction is unequivocally in the small bowel; embryonic tissues are not chalone-responsive, but clearly growth control exists in the embryo; striped muscle cells are held (wrongly) never to divide, whatever the stimulus; excessive reference is made to unpublished results, impossible to assess, including the important finding that epidermal chalone is extractable from human urine.

There is little doubt that W.S.Bullough has made seminal contributions to many facets of epidermal biology. I do not feel that the cause of the chalone concept is at all advanced by this uncritical collection of *ex cathedra* statements. There is substantial, experimental evidence for at least a negative feedback component in epithelial tissues, and my personal view is that proponents of the chalone hypothesis must provide far more substantiative evidence on isolation, bioassay, sequencing, receptor recognition, and intracellular action before any reasonably critical individual can even begin to accept many of the claims of the chalone concept.

'The evidence that this (tumour) restraining action is ... the tissue-specific chalone is now overwhelming'. To whom? What evidence? 'Toutes choses sont dites déjà; mais comme personne n'écoute, il faut toujours recommencer'. Absolument, mais au seul commencement, et ce temps, correctement.

N.A.Wright

Current Therapy in Allergy and Immunology 1983-1984. L.M.Lichtenstein and A.S.Fauci. Saint Louis: Decker. Pp. 337. Price £35.20.

This book concentrates exclusively on treatment. It contains 75 sections on diseases with prominent allergic or immunological features. Just over a third of these topics are of immediate interest to dermatologists and include common conditions such as chronic urticaria, atopic and contact dermatitis and vasculitis as well as rarities such as amyloidosis and chronic mucocutaneous candidosis. Other fields covered include immunological disorders of the joints, lungs, kidneys, liver, gastrointestinal tract and bone marrow. Each section averages 4–5 pages and the contributors, all drawn from the U.S.A., are widely recognized clinical investigators active in the study and treatment of the conditions about which they write. The idea of a book devoted to treatment methods is a good one and the editors point out that although there is a wealth of information available about the various disorders, therapy is often given rather scant attention and may be controversial. However, it is in their desire to avoid controversy that the editors have spoiled what could have been a valuable and original work. Each section is a detailed personal account of the individuals' own methods of treatment and there are no references. The result is that it is often difficult to discern which of the suggested treatments are supported by sound data, which are widely practised, but of undocumented value and which are the idiosyncrasies of the individual. A few authors are clearly unhappy about their terms of reference and the Karpatkins in particular make a valiant effort to overcome these constraints in their article on autoimmune thrombocytopenic purpura.

The editors' view that clinicians want only a didactic presentation of treatment is mistaken in my view. Most of the diseases considered will be treated by specialists in the U.K. and I cannot accept that the majority of such doctors are incapable of considering for themselves evidence for or against a particular point of view or that they should not be encouraged to do so. There is much of value to be gleaned from the book, but I hope that in future editions the editors will not underestimate either the capacity of their less illustrious colleagues to weigh conflicting views for themselves or their need for source references for the opinions presented. Even their authors' approach to treatment might benefit from such critical appraisal.

M.G.C.Dahl

Guidelines to Eczema-Dermatitis in Childhood. F.O.C.MEENAN (1983) Dublin: Mount Salus Press. Pp. 43. Price IR £4.75.

This booklet has been sponsored by some thirteen well-known pharmaceutical firms who manufacture dermatological products.

The author is a senior Dublin dermatologist whose aim is to provide an introduction to 'a difficult and complex subject for doctors in general practice, nurses and paramedical disciplines dealing with children'. There are chapters on atopic dermatitis, seborrhoeic dermatitis, contact dermatitis which includes napkin dermatitis, and a small formulary and list of references.

There are perhaps inevitably some controversial views such as 'atopic dermatitis is not a rash that itches but an itch that rashes'. The author in general terms encourages the bathing of atopic children but forbids it in seborrhoeic babies. The useful formulary recommends Silcock's base but does not divulge its contents. In the list of topical corticosteroids, hydrocortisone 17-butyrate is classified as a moderately potent rather than a potent topical steroid.

There are twenty black-and-white photographs most of which are excellent and clearly show the intended clinical features.

On the whole I enjoyed reading these guidelines which contain much good sense and sound advice.

W.N.MORLEY

Book received

Varicocele and Male Infertility. Edited by M.GLEZERMAN AND E.W.JECHT (1984) Berlin:Springer. Pp. 122. Price DM 38.00.

British Journal of Dermatology (1984) **111**, 378.

News and Notices

Paediatric Dermatology Seminar, Miami Beach, February 1985

A Paediatric Dermatology Seminar will be held at the Doral Beach Hotel, Miami Beach, Florida, on 21–24 February 1985. The Seminar fee will be U.S. $240. For further information contact Dr G.Kahn, 16800 NW 2nd Avenue, Suite 401, Miami, Florida, 33169, U.S.A.

Acknowledgment of dual publication

AOKI, K. AND SUZUKI, H. The morphology and hardness of the nail in two cases of congenital onychoheterotopia. *British Journal of Dermatology*, 1984, **110**, 717–723. Two cases described in this paper have previously been published in Japanese by the same authors in the *Japanese Journal of Dermatology*, 1983, **93**, 1167–1172. Unfortunately this was not mentioned when the paper was submitted for publication. The Editor believes that such cases of dual publication should always be acknowledged, in order to avoid possible confusion over the incidence of rare diseases, and also in order to save readers the necessity of obtaining a translation of papers in foreign languages if no new information is contained.

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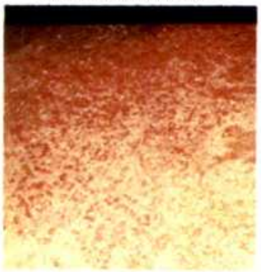
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The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

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microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
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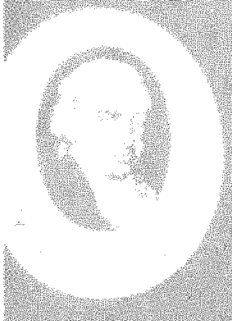

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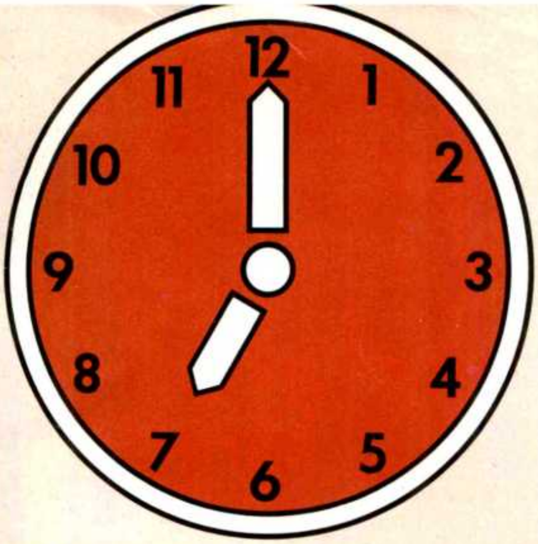

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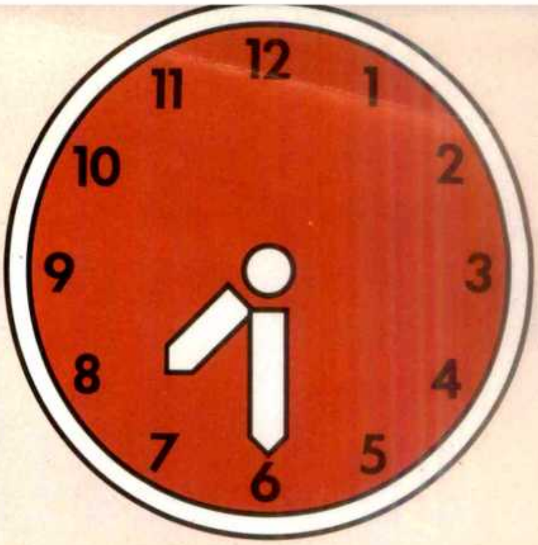

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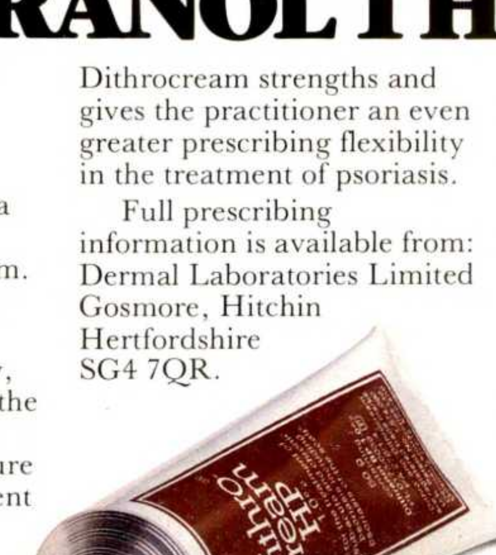

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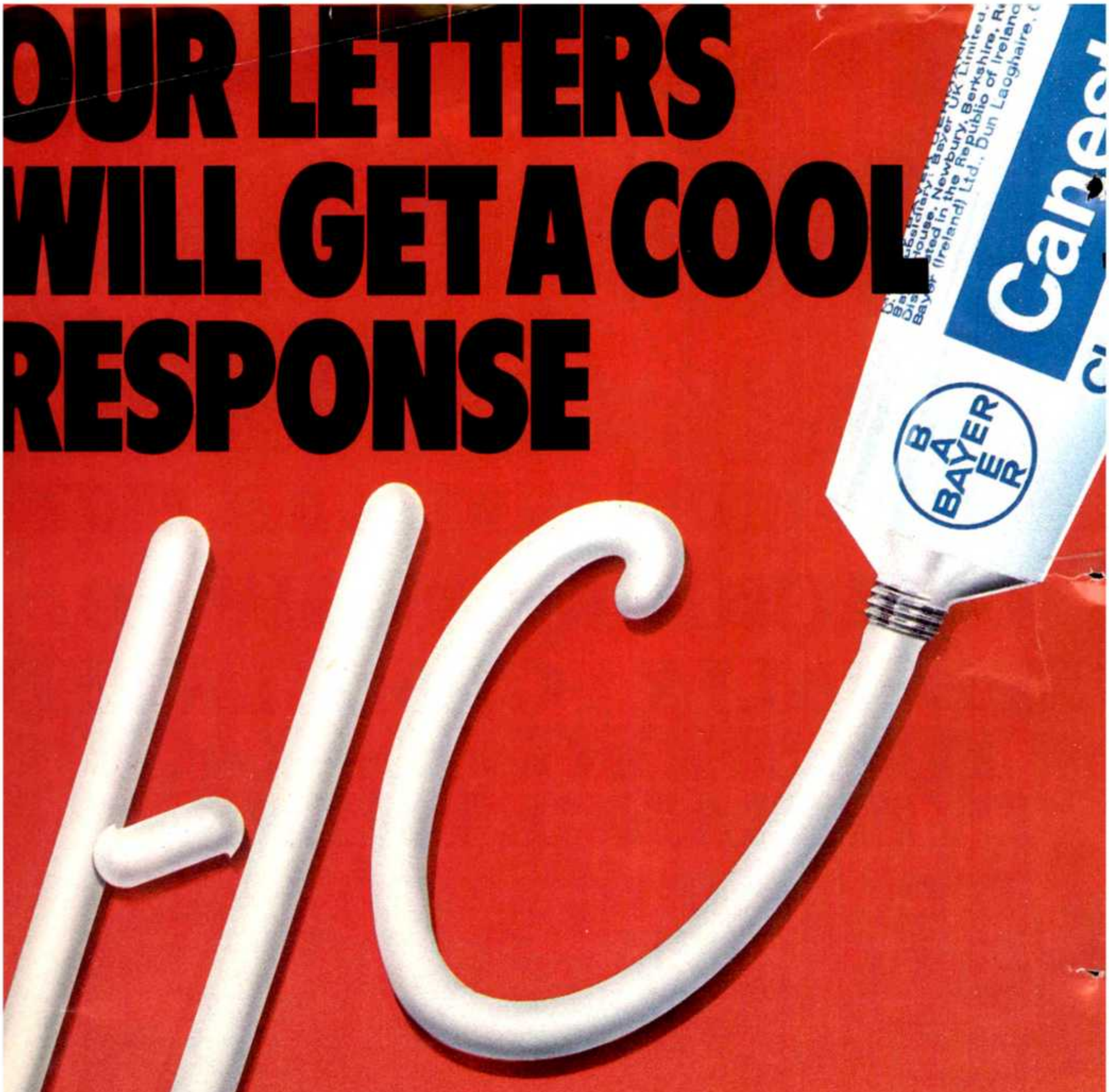

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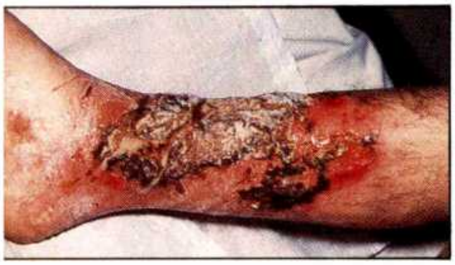
Full information and data sheet on request
Stiefel Laboratories (UK) Limited, Wellcroft Road, Slough SL1 4AQ.

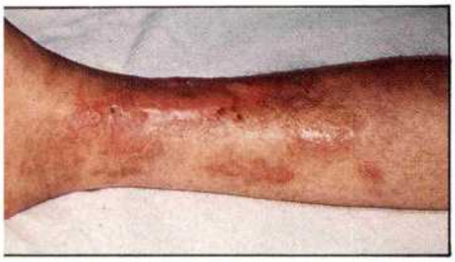
Prescribing information Stiedex: 0.25% desoxymethasone in an oily cream base; Stiedex LP: 0.05% desoxymethasone in an oily cream base: Stiedex L P N: 0.05% desoxymethasone and 0.5% neomycin (as the sulphate) in an oily cream base. **Uses:** Stiedex: treatment of severe acute inflammatory and allergic conditions; and for chronic skin disorders such as psoriasis. Stiedex L P: treatment of eczema; non-specific treatment of sunburn; insect bites, anogenital pruritis. Stiedex L P N: steroid responsive dermatoses complicated by bacterial infection. **Administration:** apply sparingly to the affected area and rub gently into the skin 2 to 3 times daily. **Contra-indications:** not suitable for infants and young children. Not to be used in primary infective diseases of the skin, in particular, tuberculous, fungal and viral infections, e.g. herpes simplex and zoster, viral warts. Do not use extensively in pregnancy, i.e., in large amounts or for long periods. Not suitable for inflammatory disorders of the eye. Stiedex L P N: not to be used in patients with a known hypersensitivity to neomycin. **Warnings:** Long-term continuous topical steroid therapy should be avoided where possible, particularly in infants and children as adrenal suppression can occur, even without occlusion. Moreover, prolonged administration of corticosteroids causes a reduction in skin collagen and subcutaneous atrophy, resulting in striae, thinning and dilation of superficial blood vessels, especially when the corticosteroid is used on the face. Stiedex, Stiedex L P oily creams may be diluted if necessary, by admixture with Aqueous Cream B P. **Legal category** POM. **Packs and NHS prices:** Stiedex, tubes of 30g: £3.43, Stiedex L P, tubes of 30g: £2.86, Stiedex L P N, tubes of 15g: £2.17. **PL no's** Stiedex – 0174/0052; Stiedex L P 0174/0053; Stiedex L P N 0174/0054.

References 1. Cutis (1978) **21,** 403 2. Curr. Med. Res. Opin (1977), **4,** 650 3. Curr. Ther. Res. (1978) **24,** 37 4. Drugs (1978) **16,** 302

Ulcer healing is



7 year old heroin addict with
xtensive infected ulceration.



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IODOSORB is easy to apply and easy to remove. At dressing change, the risks of damage to newly-formed skin are minimised.

Clinical trials have proved that the use of IODOSORB results in faster healing than standard treatment in wet exudating leg ulcers and pressure sores.[1,2,3] Pain and odour are also appreciably reduced early in treatment.

RESCRIBING INFORMATION

resentation: Sachets of sterile cadexomer iodine, a dry yellow-brown powder of odified starch gel microbeads containing iodine Ph.Eur. at a concentration of 9% w/w for topical application. **Uses:** Treatment for chronic leg ulcers sociated with venous disease. Treatment of pressure sores. **Dosage & dministration:** 'Iodosorb' is applied to the wound surface to a minimum depth 3mm and covered with a dry, sterile non-adherent dressing. The dressing ould be changed daily or when the 'Iodosorb' has become saturated with ound exudate. Each time the dressing is changed and at the end of treatment, e remaining 'Iodosorb' should be gently washed from the ulcer surface either ith a stream of sterile water or with a wet sterile swab. To avoid the risk of cross-contamination, it is recommended that a sachet of 'Iodosorb' be confined to the treatment of a single patient per day. 'Iodosorb' is a topical treatment for the wound and has no influence on the underlying disturbance of circulatory function. Where considered appropriate, support bandages or stockings can be applied in conjunction with the use of 'Iodosorb'. **Contra-Indications, Warnings etc: Contra-Indications:** As 'Iodosorb' contains 0.9% iodine, it should not be used in patients with known or suspected iodine sensitivity. **Warnings:** Iodine is absorbed systemically especially when large ulcers are treated. The use of 'Iodosorb' should be avoided in pregnant or lactating women; absorbed iodine can cross the placental barrier and is secreted into breast-milk. **Precautions:**

In wet, venous leg ulcers

IODOSORB

cadexomer iodine Stuart

fast, active healing

Systemic absorption of iodine should be taken into consideration if the thyroid function of a patient is under investigation. It has been observed occasionally that an adherent crust can form when the dressing is not changed with sufficient frequency. These crusts can be softened during dressing changes. **Side-Effects:** Some patients experience a transient smarting sensation during the first hour after application of 'Iodosorb'. Mild erythema without sensitisation has also been reported. **Pack Size & Basic NHS Price:** Box of 7 x 3g sachets. £10.95. **Product Licence Number:** 29/0165. **References:** 1. Ormiston M.C., *et al.* in Fischer H. eds Cadexomer Iodine. Stuttgart: F. K. Schattauer. 1983:63. 2. Moberg, S., *et al.* J.Amer.Geriat.Soc.1983; 31:462. 3. Skog, E., *et al.* Br. J. Dermatol 1983; 109:77.

Further information available on application to the company:
Stuart Pharmaceuticals Ltd, Carr House,
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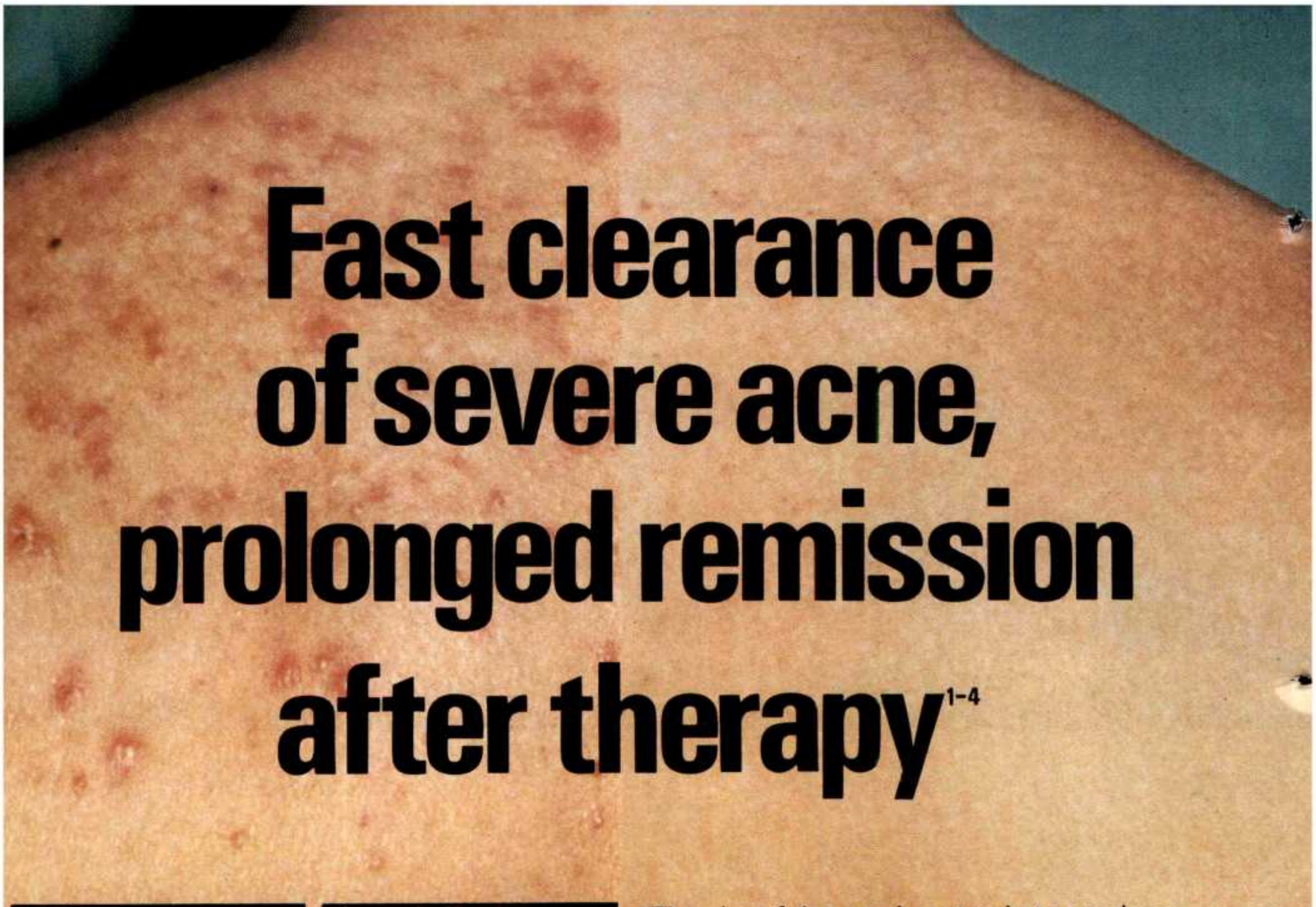
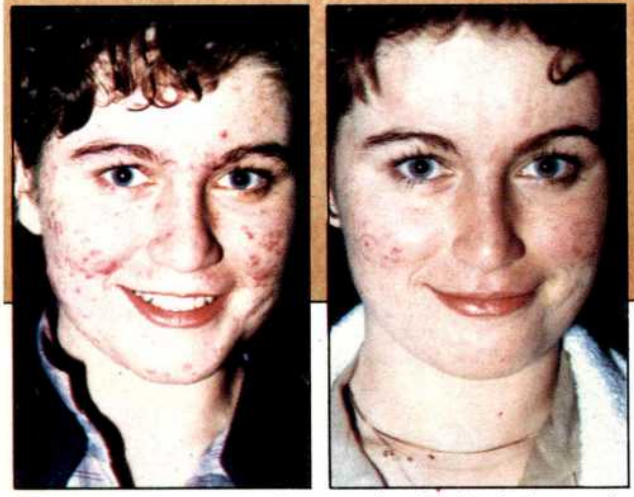


Fast clearance of severe acne, prolonged remission after therapy[1-4]



Female patient unresponsive to oral antibiotics, before and after a course of Roaccutane.

"The aim of therapy is not only to produce clearing of active disease, but also to maintain this clearing indefinitely."[5]

In severe acne, Roaccutane rapidly produces an excellent response in 90% of patients and prolonged remissions after therapy once clearance is achieved.[1-4]

(Main picture) Male patient with truncal acne unresponsive to oral antibiotics, before and after a course of Roaccutane.

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a turning point in acne therapy, for both male and female patients

BRIEF PRESCRIBING INFORMATION

Indications Cystic and conglobate acne, severe acne unresponsive to systemic antibiotics.

Dosage 0.5mg/kg daily for 12-16 weeks. Increase up to 1mg/kg daily if response is poor at 4 weeks. Reduce dosage if tolerance is poor. As a rule, other anti-acne therapy, including antibiotics, is not indicated. Repeat course of treatment should not normally be given.

Contra-indications Pregnancy (Roaccutane is teratogenic—see 'Precautions') and breast-feeding. Hepatic or renal impairment.

Precautions The following precautions must be observed in women of childbearing potential.

1. Patients should be given detailed explanation of the risks,
2. Pregnancy should be excluded before therapy is instituted.
3. Effective contraception must be ensured for one month before treatment, during treatment, and for one month after treatment ends.

Use of patient information leaflets is strongly recommended. Monitor liver function and serum lipids (fasting value) regularly.

Side-effects Mainly mucosal dryness and fissures, facial dermatitis, desquamation, epidermal fragility, conjunctivitis, arthralgia/myalgia, increased hair loss (uncommon and reversible), possible elevation of liver enzymes and serum triglycerides. Benign intracranial hypertension has been reported, particularly in association with concomitant antibiotics.

Availability Hospitals and hospital clinics.

Presentations and Basic NHS Cost

Roaccutane capsules 5mg × 100 £33.56.

Roaccutane capsules 20mg × 100 £96.73

Product Licence Numbers 0031/0158 (capsules 5mg), 0031/0160 (capsules 20mg).

Licence Holder

Roche Products Limited
PO Box 8, Welwyn Garden City
Hertfordshire AL7 3AY

References

1. In: "Retinoids: Advances in Basic Research and Therapy". Eds. Orfanos, C.E. *et al.*, Springer-Verlag, Berlin, 1981, p.219.
2. Brit.J.Derm., 1983, *108*, 333.
3. J.Amer.Acad.Derm., 1982, *6*, 735.
4. J.Amer.Acad.Derm., 1982, *6*, 766.
5. J.Amer.Acad.Derm., 1983, *9*, 629.

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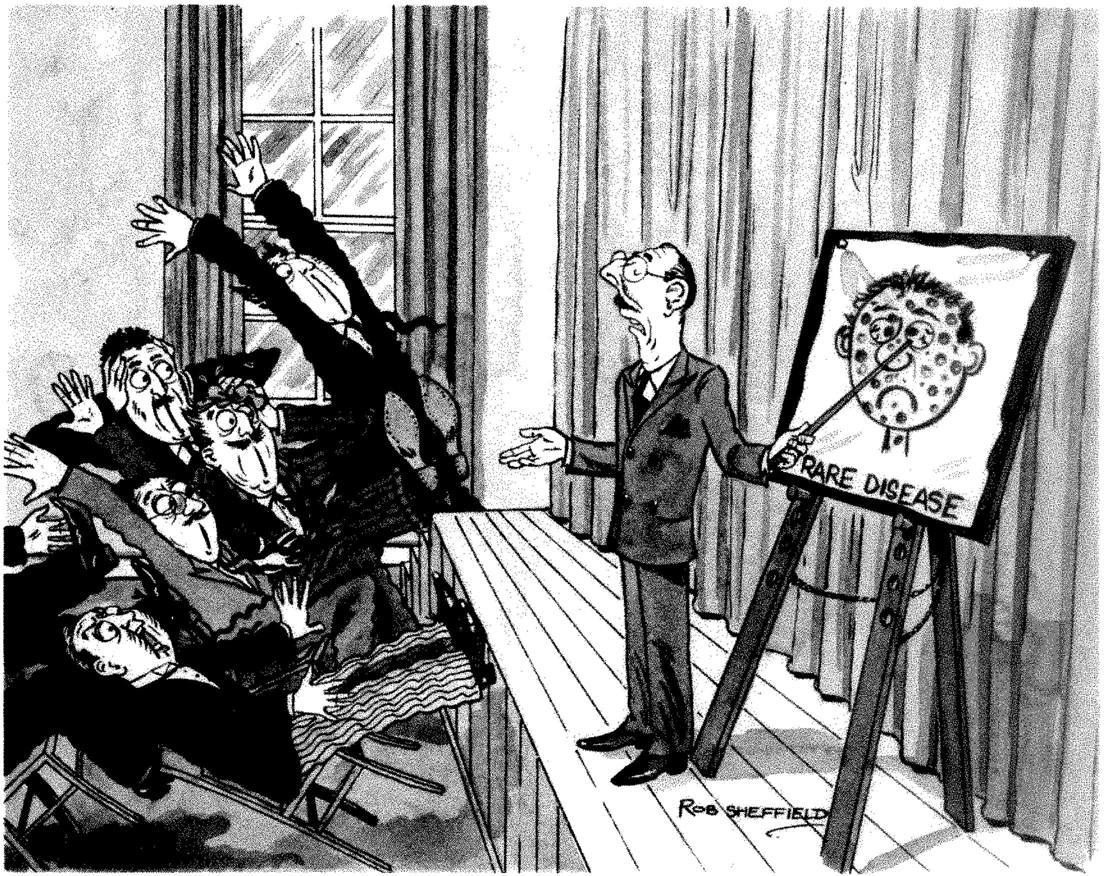

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Prescribing information

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Uses

Inflammatory dermatoses responsive to topical corticosteroid therapy. Betnovate RD preparations are indicated for maintenance treatment when control has been achieved with Betnovate (0·1%) Cream or Ointment.

Dosage and administration

A small quantity of Betnovate RD should be applied to the affected area two or three times daily. Betnovate RD Cream is especially appropriate for moist or weeping surfaces and Betnovate RD Ointment for dry, lichenified or scaly lesions, but this is not invariably so.

Precautions

Long-term continuous topical therapy should be avoided where possible, particularly in infants and children, as adrenal suppression can occur even without occlusion. Appropriate antimicrobial therapy should be used whenever treating inflammatory lesions which have become infected. Any spread of infection requires withdrawal of topical corticosteroid therapy and systemic administration of antimicrobial agents. Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Side effects

Prolonged and intensive treatment with highly active corticosteroid preparations may cause local atrophic changes in the skin such as striae, thinning and dilatation of the superficial blood vessels. As with other topical corticosteroids, prolonged use of large amounts, or treatment of extensive areas, can result in sufficient systemic absorption to produce the features of hypercorticism. This effect is more likely to occur in infants and children. Betnovate RD preparations are usually well tolerated, but if signs of hypersensitivity appear, application should stop immediately.

Contra-indications

Rosacea, acne and peri-oral dermatitis. Skin lesions caused by infection with viruses (e.g., herpes simplex, chickenpox), fungi (e.g., candidiasis, tinea) or bacteria (e.g., impetigo). Hypersensitivity to the preparation.

Product licence numbers

Betnovate RD Cream: 0004/0277

Betnovate RD Ointment: 0004/0278

Basic NHS cost (exclusive of VAT)

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100 gram tubes £2·90

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Dosage and administration: Four times a day, moisten brush with Monphytol and apply to the affected parts, reaching gently into the folds of the skin. Treatment should be repeated from time to time after the condition has subsided to prevent reinfection.

Contra-indications, warnings, etc: Monphytol may sting sensitive weeping areas of acutely inflamed skin. Other treatment (to reduce inflammation and exudation) may first be necessary. Although there is only a small amount of boric acid present, caution is advised in the repeated application of Monphytol to extensive areas of the skin.

Pharmaceutical precautions: Store away from heat.

Legal category: P.

Package quantities: Bottles containing 18 ml with brush fitted in cap.

Further information: Nil.

Product licence number: 0118/5010

Basic NHS price: £0.57 per 18 ml bottle.

Reference

1. Krebs HA, Wiggins D, Stubbs M, Sols A, Bedoya F. Studies on the mechanism of the anti-fungal action of benzoate. Biochem J. 1983; **214**: 657-63.

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Laboratories for Applied Biology 91 Amhurst Park, London N16 5DR

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dults and children over 12 years. One tablet twice daily. Minocin may be taken with eals. Treatment of acne should be continued for a minimum of six weeks.
ontra-indications
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recautions
bsorption is impaired by iron and antacids containing calcium, magnesium and luminium salts.

Warnings and side effects
Gastro-intestinal disturbances are the side effects most commonly reporte
A yellow to brown discoloration of teeth is a possible sequela of treatment du
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Basic NHS price
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1983. 156 pages, illustrated. Paper, £10.95
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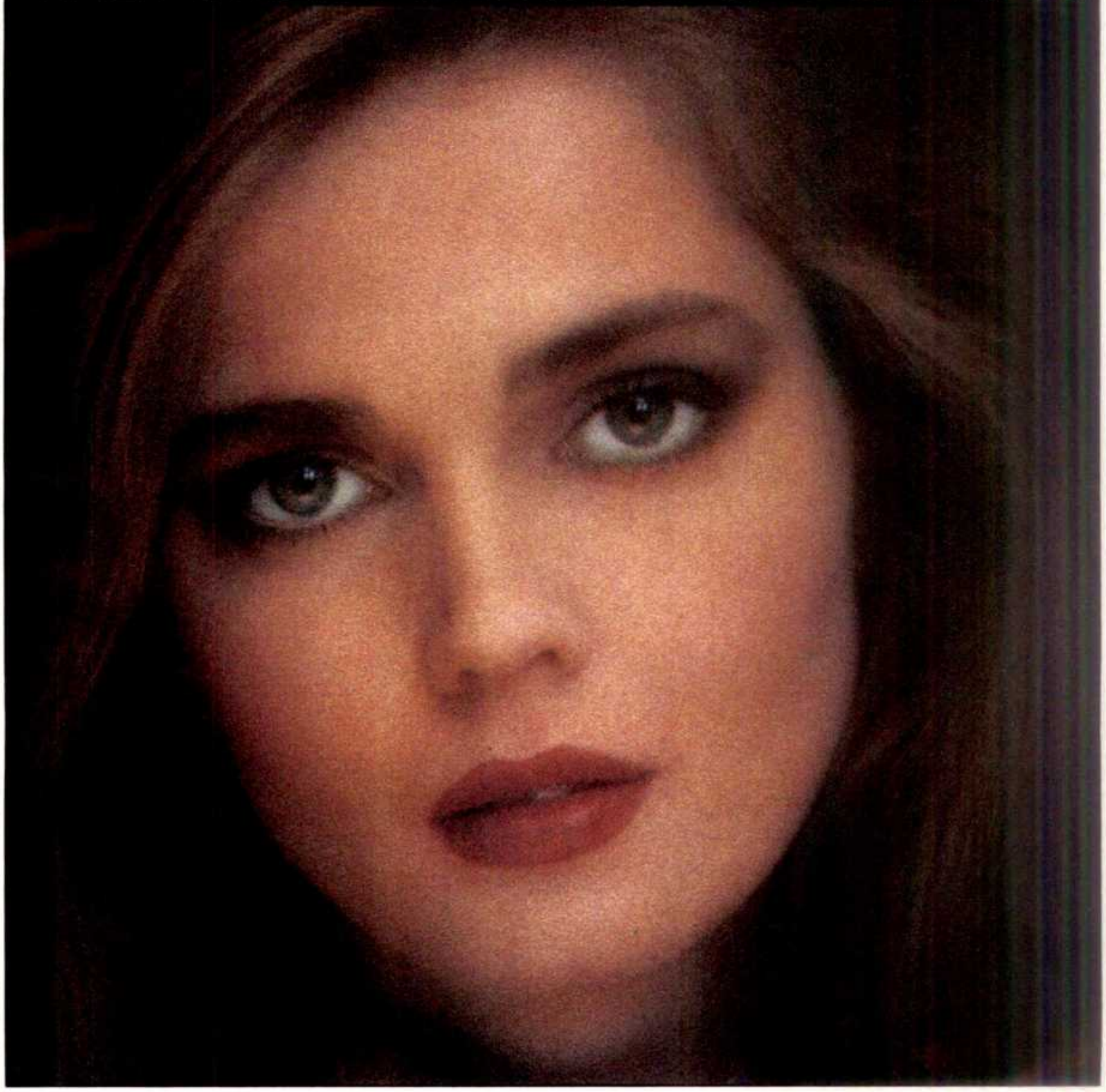

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(coal tar 5%, allantoin 2%)

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Diseases of the Hair and Scalp

Arthur Rook MA, MD, FRCP *Consultant Dermatologist, Addenbrookes Hospital, Cambridge*
and **Rodney Dawber** MA, MB, ChB, FRCP *Consultant Dermatologist, John Radcliffe Hospital, Oxford*

This important title, the first work on the subject for a number of years, will be an essential reference for all clinicians involved in the diagnosis and management of diseases concerned with the hair and scalp. It presents a comprehensive account of the hair and its disorders, emphasising their pathogenesis and diagnosis, based on a review of the international literature and the authors' extensive experience.

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1982. 567 pages, 197 illustrations. £40.00

Contents: The comparative physiology, embryology and physiology of the human hair; Hair follicle structure and keratinisation and the physical properties of hair; The hair in infancy and childhood; Hair patterns: baldness and hirsutism; Diffuse alopecia: endocrine metabolic and chemical influences on the follicular cycle; Hereditary and congenital alopecia and hypotrichosis; Defects of the hair shaft; Hypertrichosis; Traumatic alopecia; Alopecia areata; Cicatricial alopecia; Hair colour; Infections and infestations of the hair and scalp; Psychological factors and disorders of the hair; Hair cosmetics; Hair and scalp in systemic diseases; Diseases of the scalp and skin diseases involving the scalp; Naevi, tumours and cysts of the scalp; Investigation of hair and hair follicle diseases.

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ESTABLISHED: July, 1979 **PUBLISHED:** Monthly **EDITOR:** J. Graham Smith, Jr., M.D.

The **JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY** is a professional journal devoted exclusively to the clinical and continuing education needs of the entire dermatologic community, including those family practitioners, pediatricians, and internists who are concerned with clinical manifestations of skin disease in their practice.

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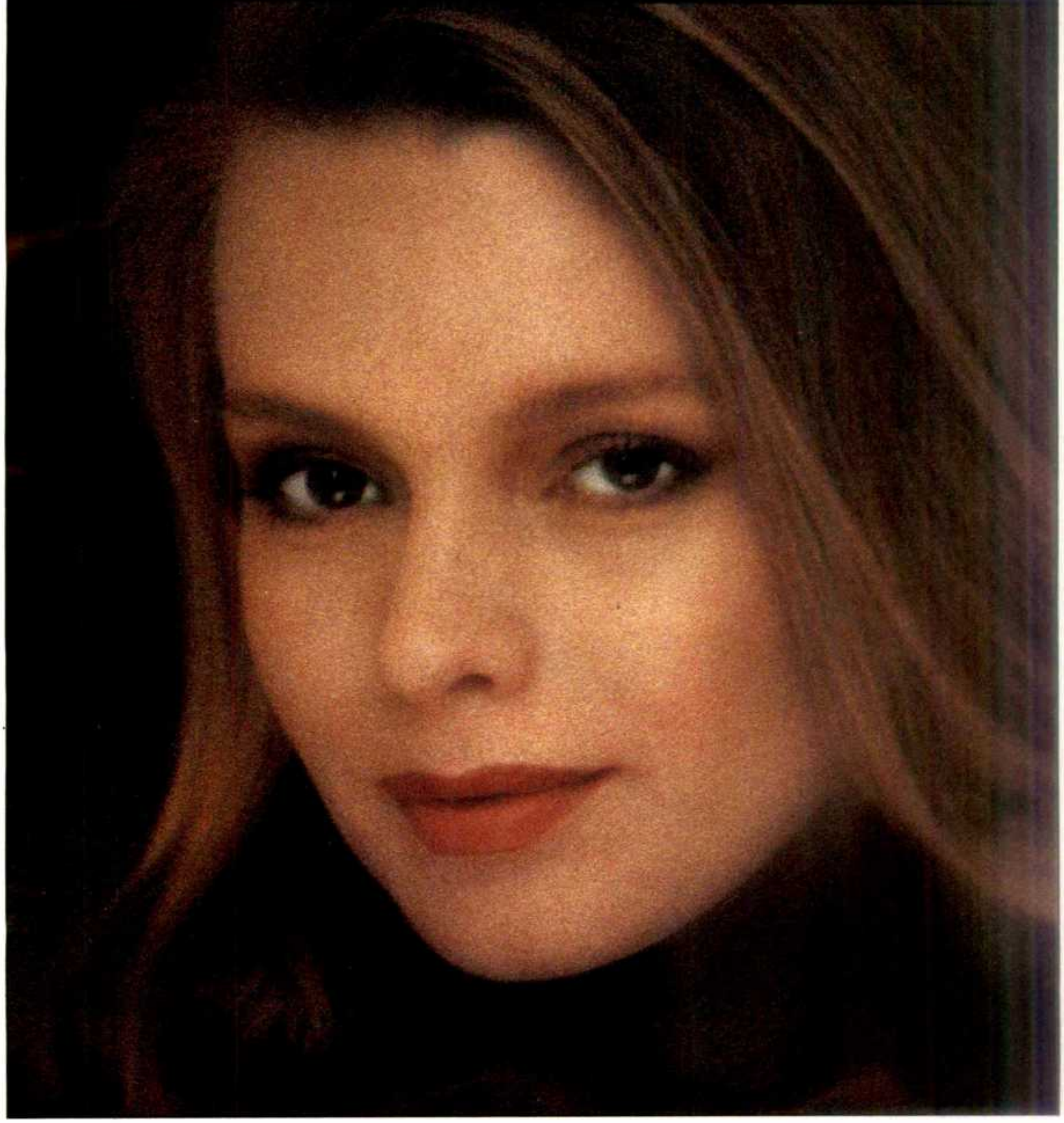

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Presentation: 50g tube **Product Licence:** 0173/0015 (PA 278/10/1)

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British Journal of Dermatology (1984) **111**, 379–387.

Clinical and Laboratory Investigations

Evidence for a functional defect of the lamina lucida in recessive dystrophic epidermolysis bullosa demonstrated by suction blisters*

M.J.TIDMAN AND R.A.J.EADY

Department of Electron Microscopy and Cell Pathology, Institute of Dermatology, London, U.K.

Accepted for publication 6 April 1984

SUMMARY

Suction blisters were raised in non-lesional skin of sixteen patients with various types of epidermolysis bullosa (EB). The ultrastructural level of separation was found in each type to be within the lamina lucida of the epidermal basement membrane zone. Suction blister times were normal in EB simplex, dominant dystrophic and localized recessive dystrophic EB, but were markedly reduced in both junctional EB and severe generalized recessive dystrophic EB.

These data indicate an unpredicted abnormality of adhesion within the lamina lucida in severe recessive dystrophic EB, in addition to the well-recognized defect at a deeper level, beneath the lamina densa, in this condition.

The use of suction blister times may prove valuable in the diagnosis of EB.

Epidermolysis bullosa (EB) is the collective term for the inherited disorders which are characterized by a tendency to blister due to a reduced tolerance to frictional trauma. There are three major variants, each with typical clinical features and a specific level of blister formation in, or immediately adjacent to, the dermo-epidermal junction (DEJ) (Bauer & Briggaman, 1979; Eady & Tidman, 1983; Fig. 1). In EB simplex (EBS) the blister forms by lysis of the basal keratinocytes leaving a variable amount of cytoplasm attached to an intact DEJ. In junctional EB (JEB) cleavage occurs within the lamina lucida (LL) of the DEJ, and in dystrophic EB (DEB) separation takes place just beneath the lamina densa in the most superficial region of the dermis. The precise mechanisms of blistering are as yet unknown for any form of EB although certain variants are associated with specific structural or biochemical abnormalities (Bauer & Briggaman, 1979; Eady & Tidman, 1983).

In normal skin, friction blisters develop within the stratum spinosum of the epidermis

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* Presented in part at the Annual Meeting of the British Society for Investigative Dermatology, University of Nottingham, 1983.

Correspondence: Dr R.A.J.Eady, Department of Electron Microscopy and Cell Pathology, Institute of Dermatology, Homerton Grove, London E9 6BX, U.K.

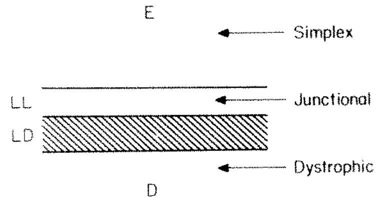

FIGURE 1. The level of natural blister formation in the three major variants of epidermolysis bullosa. E: epidermis. D: dermis. LL: lamina lucida. LD: lamina densa.

(Naylor, 1955; Sulzberger *et al.*, 1966; Hunter, McVittie & Comaish, 1974b). In all three major types of EB, experimental friction blisters and natural lesions arise at the same ultrastructural level (Pearson, 1967). However, the effect of a suction force on cleavage levels in EB is not known, although in normal skin, suction will consistently induce dermo-epidermal separation within the LL (Kiistala & Mustakallio, 1967; Hunter, McVittie & Comaish, 1974a). Furthermore, the time taken for a suction blister to develop has been used as a measure of the cohesive forces within the LL (Kiistala, 1972a,b), and may thus provide valuable functional information about the DEJ. Previous reports have suggested that the suction blister times (SBT) in epidermolysis bullosa of unstipulated type (Slowey & Leider, 1961) and in recessive DEB (Bielicky, 1956) are reduced.

The aims of this study were to determine the ultrastructural level at which suction blisters form in the various types of EB and to measure the speed of their formation.

METHODS

Sixteen patients with EB were studied, and most of the major types were represented (Table 1). The diagnoses were made on the basis of clinical, genetic and ultrastructural criteria. Two subjects had a generalized form of EBS, one having the herpetiform clustering of blisters and palmoplantar hyperkeratosis characteristic of the Dowling–Meara variant (Dowling & Meara, 1954), although the tonofilament changes described in this condition (Anton-Lamprecht & Schnyder, 1982) were not observed. The four patients with JEB included two middle-aged females with clinical features typical of the non-lethal form (Hintner & Wolff, 1982) and in whom morphometric studies had demonstrated the presence of numerically and structurally normal hemidesmosomes (Tidman & Eady, 1983a). The third JEB subject was a 6-year-old boy with a severe form of the condition and in whom the hemidesmosomes lacked normal sub-basal dense plates. The fourth JEB subject was a female aged 20 years with relatively mild disease, who was clinically difficult to classify as she had features consistent with both DEB and JEB. Microscopy of a natural blister, however, confirmed junctional separation. The four patients with a mild localized form of recessive DEB were two pairs of siblings with no other member of either family affected, and they were clinically indistinguishable from the three subjects with dominant dystrophic EB, all of whom had affected relatives in two or more generations. The three patients with severe generalized recessive DEB had the typical mutilating clinical features of this condition (Bauer & Briggaman, 1979).

The control group consisted of eight female and five male healthy volunteers, aged 22 to 57 years. Apart from three children with EB, appropriate age and sex controls were present for each EB subject.

TABLE 1. Subject details and ultrastructural localization of natural and suction blisters

	Clinical diagnosis	Age (years)	Sex	Level of natural or friction blister	Level of suction blister
Controls		22–57	8F:5M	ND	LL
Patients					
W.F.	EBS (Dowling–Meara)	39	F	Basal epidermis	LL
K.C.	Generalized EBS	30	M	Basal epidermis	LL
E.B.	Non-lethal JEB	61	F	LL	LL
J.M.	Non-lethal JEB	51	F	LL	ND
A.A.	Non-lethal JEB	20	F	LL	ND
M.M.	Severe JEB	6	M	LL	ND
J.H.	DDEB—mild, localized	41	F	ND	LL
K.B.	DDEB—severe, generalized	61	M	ND	LL
M.R.	DDEB—moderate, localized	26	M	ND	ND
M.D.	Mild, localized RDEB	57	F	Sub-LD	LL
B.B.	Mild, localized RDEB	61	F	Sub-LD	LL
I.H.	Mild, localized RDEB	12	M	ND	LL
N.H.	Mild, localized RDEB	7	M	ND	ND
M.H.	Severe RDEB	36	F	Sub-LD	LL
P.A.	Severe RDEB	36	F	Sub-LD	LL
J.D.	Severe RDEB	16	F	ND	ND

EBS = epidermolysis bullosa simplex.
JEB = junctional epidermolysis bullosa.
DDEB = dominant dystrophic epidermolysis bullosa.
RDEB = recessive dystrophic epidermolysis bullosa.
LL = lamina lucida.
LD = lamina densa.
ND = not done.

Suction blister technique and electron microscopy

Suction blisters were raised in four body regions, namely the lower half of the anterior abdominal wall and the inner aspects of the upper arm, thigh and lower leg, using a standard suction cup (Kiistala, 1968) containing a plastic diaphragm perforated by five holes of 8 mm diameter. The cups were connected to a vacuum pump (Eschmann Matburn DV20) set to deliver a suction pressure of 200 mmHg below atmospheric. This pressure was chosen because it induced blistering within a convenient time in control subjects. The SBT was defined as the time taken for the first sign of a vesicle to appear in any of the circles of skin outlined by the holes in the diaphragm. If blister formation had not occurred after 3 h the experiment was terminated, and the SBT recorded as 180 min. On completion, the temperature of the skin surface under the suction cup was measured with an electric thermometer (Light Laboratories, Brighton, Sussex).

In representatives of each EB type, suction blisters were allowed to grow, and the roof and floor were biopsied to determine the level of cleavage. Samples were fixed in 2% formaldehyde and 2·5% glutaraldehyde in 0·1 M cacodylate buffer (pH 7·4) with 0·05% calcium chloride and 5% sucrose for 3–4 h at room temperature (Karnovsky, 1965). The tissue was post-fixed in 1·3% osmium tetroxide buffered with 0·067 M s-collidine (pH 7·4) for 2 hours on ice, dehydrated in a graded ethanol series, and embedded in Epon 812 via propylene oxide. Ultrathin sections were double-stained with a 6% aqueous solution of uranyl acetate (pH 4·5) for 15–25 min at room temperature and lead citrate (Reynolds, 1963) for 5–8 min, and viewed with a JEOL 100 CX electron microscope.

RESULTS

Electron microscopic observations on the level of cleavage and SBT in patients and controls are detailed in Tables 1 and 2, and the mean abdominal SBT for each EB type is represented graphically in Fig. 2.

TABLE 2. Regional suction blister times in epidermolysis bullosa variants

Diagnosis		Suction blister times (min)[a]			
		Abdomen	Arm	Thigh	Lower leg
Controls		82·6 ± 33·0 (s.d.)	82·9 ± 27·4 (s.d.)	91·5 ± 27·4 (s.d.)	145·1 ± 38·8 (s.d.)
Patients					
W.F.	EBS (Dowling–Meara)	90 (34·0)[b]	62 (35·5)	73 (33·0)	130 (32·5)
K.C.	Generalized EBS	74 (33·4)	74 (34·6)	53 (33·5)	180 (28·5)
E.B.	Non-lethal JEB	5 (34·5)	5·5 (35·4)	6 (33·3)	16 (32·2)
J.M.	Non-lethal JEB	10 (32·5)	18·5 (32·0)	14·5 (30·8)	—
A.A.	Non-lethal JEB	3 (34·1)	4 (34·3)	4 (34·5)	4 (33·9)
M.M.	Severe JEB	1	—	2·5	—
J.H.	DDEB—mild, localized	132 (32·9)	116 (34·2)	138 (32·8)	180 (29·8)
K.B.	DDEB—severe, generalized	77·5 (35·3)	60 (35·0)	59·5 (34·0)	—
M.R.	DDEB—moderate, localized	115 (34·8)	70 (34·8)	95 (34·5)	—
M.D.	Mild, localized RDEB	78·5 (34·3)	41·5 (34·5)	54 (33·0)	54 (31·5)
B.B.	Mild, localized RDEB	46 (34·9)	30·5 (33·0)	62·5 (33·7)	86 (30·0)
I.H.	Mild, localized RDEB	80 (35·5)	63 (34·8)	94·5 (34·1)	86 (34·5)
N.H.	Mild, localized RDEB	101 (36·7)	84 (36·0)	105 (35·0)	146 (35·2)
M.H.	Severe RDEB	12·5 (34·4)	13·5 (35·7)	11·5 (33·6)	14 (33·2)
P.A.	Severe RDEB	16·5 (34·5)	—	22·5 (34·5)	21 (35·0)
J.D.	Severe RDEB	12 (35·7)	13 (35·3)	21·5 (35·9)	21 (34·2)

[a] Suction pressure of 200 mmHg below atmospheric.

[b] Skin surface temperature (°C) in parentheses.

SBT were normal in patients with EBS, dominant DEB and localized recessive DEB, and the normal regional variation was preserved (Table 2). In patients with dominant and localized recessive DEB there was no apparent abnormality of the speed of suction blister formation in the lower leg, which is a predilection site for natural blisters. Subjects with the severe generalized form of recessive DEB and all those with JEB blistered very rapidly in each body region tested.

Electron microscopy demonstrated that, in all variants of EB, suction blisters formed within the lamina lucida (Table 1; Figs 3 and 4).

Paranuclear vacuolization in the keratinocytes was evident in the roofs of suction blisters in all types of EB, and melanocytes were occasionally present on the floor of the suction blister, remaining in contact with the lamina densa.

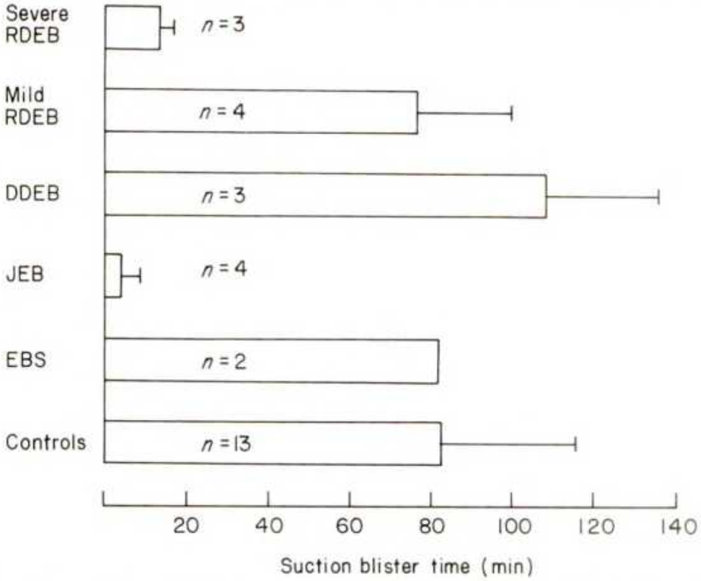

FIGURE 2. Mean abdominal suction blister times (± standard deviation) in epidermolysis bullosa variants, using a pressure of 200 mmHg below atmospheric. EBS: epidermolysis bullosa simplex. JEB: junctional epidermolysis bullosa. DDEB: dominant dystrophic epidermolysis bullosa. RDEB: recessive dystrophic epidermolysis bullosa.

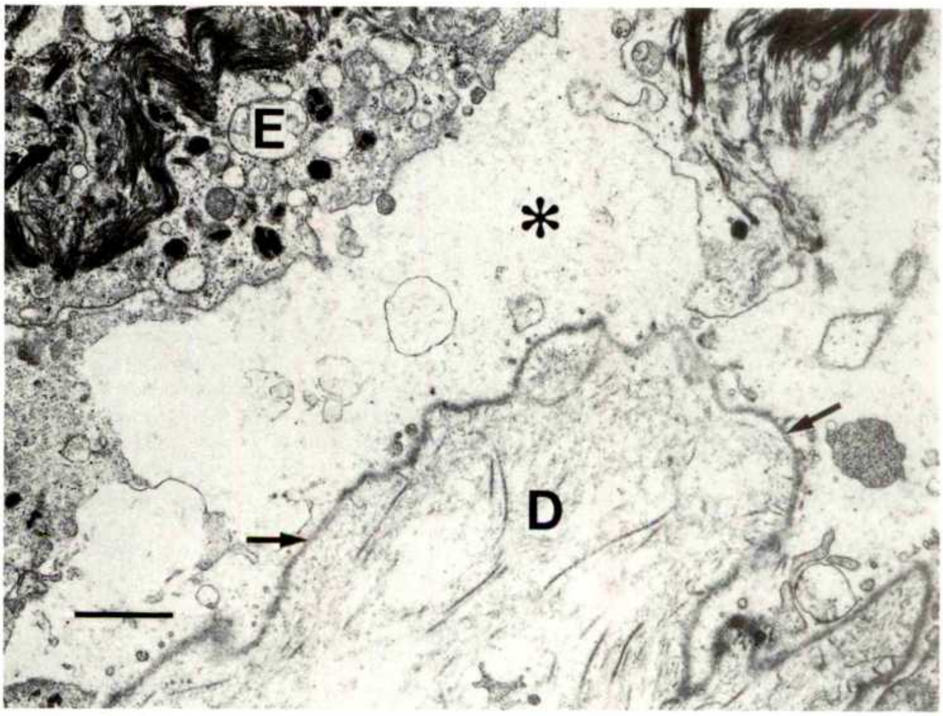

FIGURE 3. Suction blister raised on normal skin showing the epidermis (E) to have separated from the dermis (D) in the lamina lucida, leaving the lamina densa (arrowed) in the floor of the blister cavity (*). Calibration bar = 1 μm.

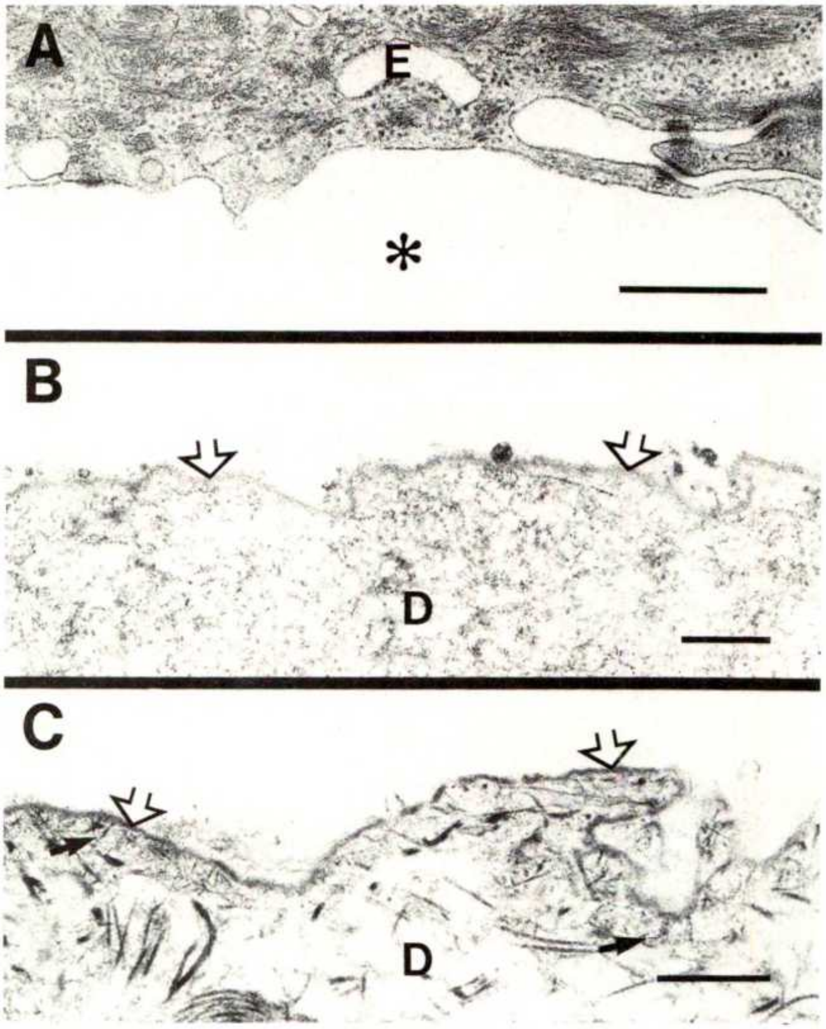

FIGURE 4. (A) Basal keratinocyte (E) in roof of suction blister from a subject with severe generalized recessive dystrophic EB, showing that the blister cavity (*) has formed within the lamina lucida. (B) Floor of suction blister in severe generalized recessive dystrophic EB, confirming dermo-epidermal separation in the lamina lucida, just above the lamina densa (arrowed), with an absence of anchoring fibrils in the dermis (D). (C) Floor of suction blister from a subject with EB simplex, showing cleavage to have occurred in the lamina lucida, with no cytoplasmic fragments attached to the lamina densa (open arrows). Numerous anchoring fibrils (closed arrows) are present in the dermis (D). Calibration bars = 0·5 μm.

DISCUSSION

A number of intrinsic and extrinsic factors influence SBT, including age, sex, body region, skin temperature and suction pressure (Kiistala, 1972a,b; Lowe & van der Leun, 1968; Peachey, 1971a,b; van der Leun, Lowe & Beerens, 1974). Increasing skin temperature by 1°C reduces blistering time by about 13% (van der Leun *et al.*, 1974), which must be considered in the present study where skin temperatures could not be controlled. However, even when allowance is made for the influence of variation in skin temperature on SBT, blistering occurred abnormally quickly in patients with JEB and severe recessive DEB.

The unexpected finding of cleavage within the LL in severe recessive DEB, together with the very rapid SBT, indicates an abnormality of the LL in addition to the recognized weakness beneath the lamina densa associated with an absence of anchoring fibrils (Briggaman & Wheeler, 1975; Tidman & Eady, 1983b; Goldsmith & Briggaman, 1983). The LL is a product of the epidermis (Briggaman, Dalldorf & Wheeler, 1971), and therefore these results suggest that severe recessive DEB may not be entirely a dermal disorder. The reduced cohesion within the LL in severe recessive DEB is probably due to a biochemical abnormality, but may also be consistent with the anchoring fibril defect. In normal skin anchoring fibrils appear to extend into the LL and become continuous with anchoring filaments (Susi, Belt & Kelly, 1967; Eady & Tidman, unpublished observations), raising the possibility that anchoring fibrils contribute to cohesion within the LL. In support of this proposal Tidman *et al.* (1983) found a degree of correlation (not statistically significant) in the regional variation of SBT and anchoring fibril numbers. In JEB, however, both friction and suction result in a split in the LL, and the anchoring fibrils are normal.

The rapid SBT in JEB reflect the reduced cohesion in the LL for which a hemidesmosome structural abnormality has been held responsible (Hashimoto *et al.*, 1976). The possibility that hemidesmosomes contribute to dermo-epidermal attachment and that an abnormality of these structures may relate to a weakness in the LL is attractive. The observation of structurally normal hemidesmosomes (Tidman & Eady, 1983a) in the presence of grossly reduced SBT in two of three cases with non-lethal JEB, however, argues against a significant role for hemidesmosomes in dermo-epidermal adhesion in this variant of EB.

The measurement of SBT, which does not require elaborate equipment or great expertise, may prove useful in the diagnosis of atypical cases of EB. This was demonstrated in the present study by subject A.A., a physically well-developed 20-year-old female who had clinical features suggestive of mild DEB, namely superficial scarring over the lower legs and typically dystrophic nails. However, blisters elsewhere healed with very little scarring and without milia. Furthermore, her teeth were healthy and she had no hair loss, in contrast to the two adults known to have non-lethal JEB. Her SBT were very abnormal (Table 2), and the provisional diagnosis of JEB made on this basis was later confirmed by electron microscopic examination of a natural blister.

The formation of suction blisters within the LL, not only in normal skin but also in all the major types of EB as shown in this study with their various adhesion defects supports the concept of the LL as the *locus minoris resistentiae* of the DEJ (Klein *et al.*, 1983). The reason why suction and friction forces produce different types of injury to the skin is not well understood, but presumably is related to the unidirectional nature of a suction force compared with the multidirectional shearing effect of friction.

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Immunohistochemical localization of epidermal basement membrane laminin and type IV collagen in bullous lesions of dermatitis herpetiformis

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SUMMARY

Antibodies against the human basement membrane proteins, laminin and the 7-S domain of type IV collagen, were used to study the epidermal basement membrane in lesional skin from four patients with dermatitis herpetiformis. The staining pattern of both antigens was mostly fragmented and sometimes absent on papillary microabscesses, but when present it was attached to the epidermal basal cells. On papillary microblisters and larger blisters the staining of both antigens showed discontinuities and was located in the floor of the blister, except for two cases where tiny fragments of laminin staining were also seen in the roof of larger blisters. These results suggest that blister formation in dermatitis herpetiformis takes place between the epidermal basal cells and the basement membrane.

The exact location of the blisters in dermatitis herpetiformis (DH) is a matter of controversy. Light microscope studies using either the periodic acid–Schiff method or silver staining have indicated that the epidermal basement membrane (BM) is either destroyed or attached to the floor of the blister (MacVicar, Graham & Burgoon, 1963; Eng & Moncada, 1974). On the other hand, most electron microscope studies (for review see Alexander, 1975) and an immunofluorescence study with sera from patients with bullous pemphigoid (Hertz, Gazze & Katz, 1976) have indicated that the splitting takes place between the BM and the dermis.

The chemical constituents of the different layers of the BM have been well characterized in recent years (for reviews, see Risteli & Risteli, 1981; Stanley *et al.*, 1982). The scaffold of the lamina densa is formed by type IV collagen, which is organized into a net-like structure (Timpl *et al.*, 1981) with the individual molecules linked to each other at one end to form a particularly resistant structure known as the 7-S collagen domain of type IV collagen (Timpl *et al.*, 1981). Among the non-collagenous components of BM, which are probably all located mostly in the

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TABLE 1. Clinical data and immunofluorescence findings

Case no.	Age at clinical diagnosis/Sex	Duration of DH (years)	Response to dapsone	Response to gluten-free diet	Immunofluorescence for IgA*
1	26/M	21	+	ND	ND
2	20/M	3	+	+†	+
3	52/F	6	+	+	+
4	18/M	3	+	+	+

ND = not done.
* All patients examined showed granular fluorescence on papillary tips.
† Worsening of DH on reintroduction of gluten.

lamina lucida layer, are a large glycoprotein called laminin, a heparan sulphate-containing proteoglycan, entactin, and at least in many BMs, fibronectin (Risteli & Risteli, 1981; Stanley *et al.*, 1982; Martinez-Hernandez & Amenta, 1983). Laminin, at least, is stained with the periodic acid–Schiff procedure (Timpl *et al.*, 1982), and it is this glycoprotein that is most probably involved in attaching the epidermal cells to the BM (Terranova, Rohrbach & Martin, 1980).

The present study was undertaken to find out whether the formation of the blisters in DH takes place above or beneath the epidermal BM. The problem was approached by staining lesional skin with antibodies against two chemically well-characterized components of the BM, laminin and the 7-S collagen domain of type IV collagen.

METHODS

We studied four cases of DH in which the skin biopsy was representative with respect to the bullous lesions. Clinical data and the results of conventional direct immunofluorescence with anti-human IgA performed on frozen sections are given in Table 1. All the three cases with

FIGURE 1. Direct immunofluorescence with an anti-IgA antibody in case 2. Granular IgA deposits are distributed in the dermal papillae, with fewer granules beneath the epidermal rete ridges.

FIGURE 2. Immunoperoxidase staining with anti-human laminin P1 antibodies (case 3). A papillary microblister (B) shows an almost continuous staining on the floor with a clear space between it and the epidermis. On a microabscess the staining of the BM is more discontinuous (arrow), but attached to the epidermis.

FIGURE 3. Immunoperoxidase staining with anti-human laminin P1 antibodies (case 2). The fragmented staining of BM is situated on the floor of the blister (arrow). The BM on the papillary microabscess is more discontinuous (arrowhead).

FIGURE 4. Immunoperoxidase staining with antibodies against the 7-S domain of human type IV collagen (case 2, section adjacent to Fig. 3). The staining of the 7-S collagen is located on the floor of the blister (arrow), and has disappeared from the papillary microabscess (arrowhead).

FIGURE 5. Immunoperoxidase staining with anti-human laminin P1 antibodies (case 3). Thin fragments of BM staining are left on the floor (arrow) and on the roof of the blister (arrowhead).

FIGURE 6. Immunoperoxidase staining with anti-human laminin P1 antibodies (case 2). Low-power view of a blister. The staining of BM is located on the floor and is fragmented and thinner than in non-bullous skin. The BMs of the dermal capillaries show intensive staining.

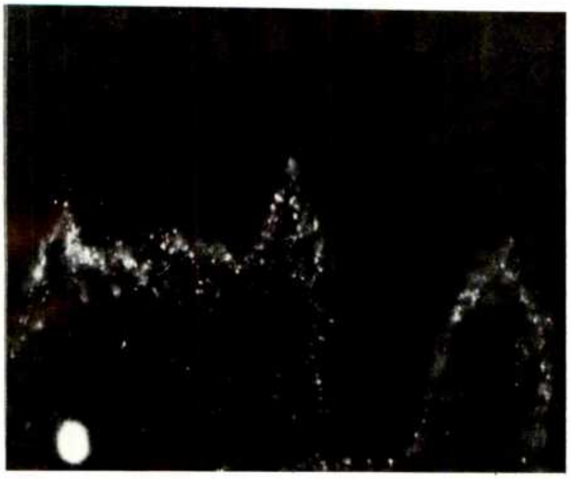


FIGURE 1

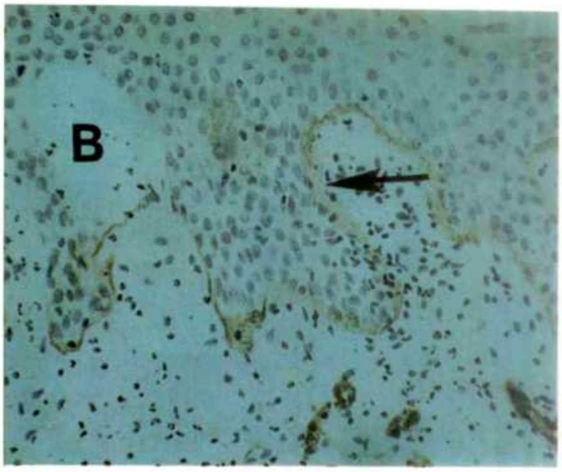


FIGURE 2

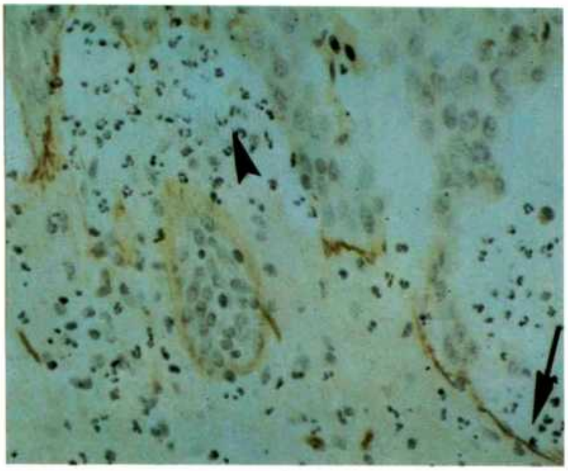
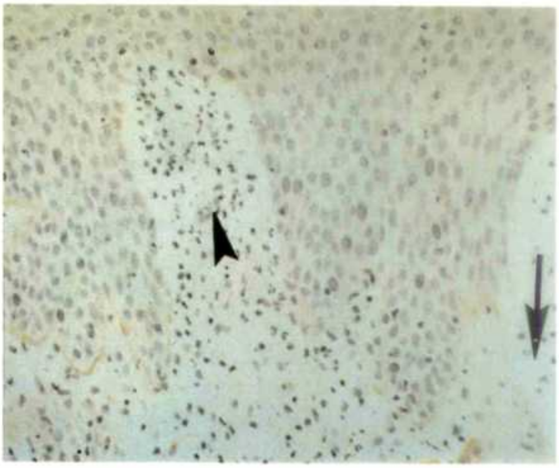


FIGURE 3



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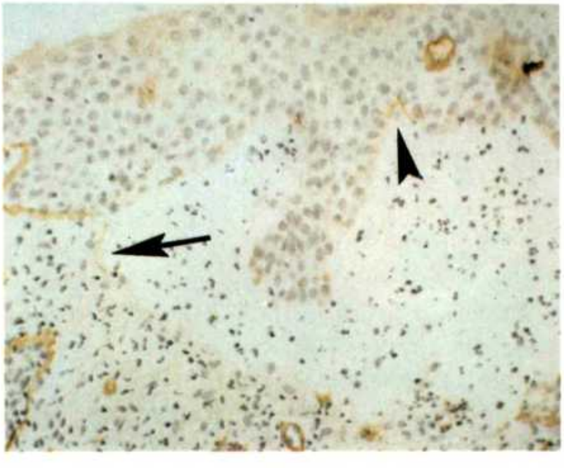

FIGURE 5

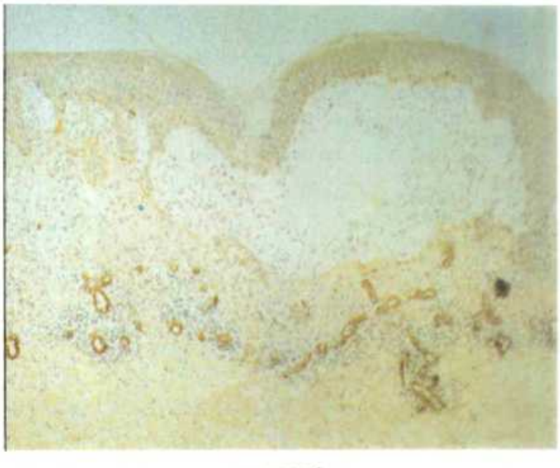

FIGURE 6

technically satisfactory sections showed granular deposits of IgA (Fig. 1). In one case (No. 2) indirect immunofluorescence was also performed, but no BM zone antibodies were detectable in the patient's serum. The DH lesions at their various stages of maturity were classified into microabscesses, microblisters and blisters according to Alexander (1975).

The 7-S collagen domain of human type IV collagen was purified from human kidney (Risteli *et al.*, 1980) and the fragment P1 of laminin from human placenta as described previously (Risteli & Timpl, 1981), and antisera to these proteins were raised in rabbits. The antibodies were purified by immunoabsorption on the relevant antigen coupled to Sepharose 4B. The laminin P1 antibodies were cross-absorbed with 7-S collagen and vice versa. There was no cross-reaction between the two antibodies in the radioimmunossay.

For immunohistochemical staining, skin biopsies from spontaneous lesions were fixed in 10% formaldehyde buffered with phosphate, and embedded in paraffin. Sections of 4 μm were deparaffinized, digested with 0·4% pepsin (Sigma Chemical Co., St Louis, Mo.) as described by Burns, Dixon and Woods (1980), and then stained using the peroxidase–antiperoxidase method (Sternberger, 1979) on glue-treated slides (Järvinen & Rinne, 1983). The concentration of the primary antibodies was 5–20 μg/ml. Normal rabbit serum and phosphate-buffered saline were used instead of the primary antibody for control stainings.

RESULTS

The staining of the epidermal BM with laminin P1 and 7-S collagen antibodies was sharp and continuous in the non-inflamed areas of the specimens, while in the inflamed areas the staining pattern varied with the phase of the lesion, but the changes were comparable in all cases.

In papillary microabscesses with relatively few inflammatory cells the staining was of normal intensity, nearly always continuous and attached to the epidermal basal cells (Fig. 2), whereas in more cellular microabscesses the staining pattern of the BM was more often fragmented and sometimes not visible at all (Figs 3 and 4). There were no differences between laminin and type IV collagen in this respect. The BM staining was either discontinuous or absent in microblisters, but when present it was always seen on the floor of the lesion (Fig. 2). The 7-S collagen staining in blisters was invariably situated on the floor (Fig. 4). Laminin staining was located exclusively on the floor of the lesion in cases 2 and 4 (Figs 3 and 6), and most of the laminin staining was seen on the floor in cases 1 and 3 as well, but with tiny fragments on the roof of the lesions (Fig. 5). In all cases the staining pattern of the BM was more fragmented and thinner in the blisters than in non-bullous skin.

Staining for both BM antigens was also seen on the BM of dermal capillaries (Fig. 6), around cutaneous appendages and on arrector pili muscles, lipocytes and smooth muscle cells in the media of the arteries (not shown), as reported previously (Weber *et al.*, 1982). The control stainings with non-immune rabbit serum and phosphate-buffered saline were negative.

DISCUSSION

In the present study we have used antibodies to chemically well-characterized basement membrane proteins to demonstrate the changes that take place in the lesions of dermatitis herpetiformis. The results suggest that the blister space forms between the epidermal basal cells and the BM, a finding which is in agreement with earlier studies using conventional light microscope histochemistry (MacVicar *et al.*, 1963; Eng & Moncada, 1974). This is also consistent with the fact that laminin stains with the periodic acid–Schiff method (Timpl *et al.*, 1982). Our findings differ from the results obtained by electron microscopy by most authors (reviewed by Alexander, 1975) but are in agreement with observations by Bellone and Caputo (1962) and Rodrigo (1972), who noticed that cleavage through the lamina lucida may occur. The discrepancy may be due to the fact that much larger specimens can be examined by light microscopy than by electron microscopy, and thus the findings of the latter may not be so representative. Another possibility is that the BM could retain its antigenicity and be visualized by immunohistochemical methods even when its ultrastructure has been unrecognizably injured.

In previous studies in which the BM antigen, recognized by sera from bullous pemphigoid patients, was used as a BM indicator this antigen could be visualized on the roof of the blisters in DH (Hertz *et al.*, 1976; Klein *et al.*, 1983). The bullous pemphigoid antigen and laminin have been identified in the lamina lucida layer of the epidermal BM, but the former is situated closer to the basal cells (Stanley *et al.*, 1982). If we assume that the DH cases studied previously (Hertz *et al.*, 1976; Klein *et al.*, 1983) represent the same disease process as our cases, it would seem that in DH the cleavage takes place and the blister is formed beneath the bullous pemphigoid antigen, but above or through laminin. A similar location between these two antigens has been reported for the lesions in junctional epidermolysis bullosa and for suction blisters (Stanley *et al.*, 1982).

Several studies have indicated preferential locations for various basement membrane components in the different layers of the membrane, type IV collagen being situated in the lamina densa and the other components mainly in the lamina lucida (Yaoita, Foidart & Katz, 1978; Foidart *et al.*, 1980; Courtoy, Timpl & Farquhar, 1982). It has also been reported, however, that at least in some BMs, all the known components are concentrated in the lamina densa layer (Laurie, Leblond & Martin, 1982). Our present results indirectly indicate that there is some difference in the distribution of laminin and type IV collagen in the epidermal BM, because some fragmented laminin was also seen on the roof of the blisters in two cases, whereas type IV collagen staining was always confined to the floor of the lesion.

The mechanism of blister formation in DH is not known in detail, although it has been suggested that papillary deposition of IgA leads to complement activation (Katz & Strober, 1978). Our results indicate that BM components are destroyed during blister formation. Neutrophilic leukocytes are known to contain enzymes that are capable of degrading type IV collagen and laminin (Mainardi, Dixit & Kang, 1980; Uitto, Schwartz & Veis, 1980). It seems that in DH the two BM components laminin and type IV collagen are antigenically changed or destroyed.

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IgA anti-endomysium antibody. A new immunological marker of dermatitis herpetiformis and coeliac disease

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SUMMARY

The recently described IgA anti-endomysial antibodies (IgA-EmA) are directed against the intermyofibril substance of the smooth muscle, which may correspond either to a reticulin-like structure or a surface component of smooth muscle fibrils. These antibodies occurred in about 80% of sera of thirty-eight patients with dermatitis herpetiformis (DH), in about 70% of twenty-eight patients with coeliac disease and in about 20% of nine patients with other enteropathies. IgG class anti-gliadin antibodies (AGA) also occur in each of these diseases. Both antibodies were detected on monkey oesophagus by immunofluorescence. The IgA-EmA could not be detected in 122 control sera from patients with other gut or skin diseases, including fifteen cases with ulcerative colitis and fifteen cases with linear IgA bullous dermatosis (LABD). The presence and the titre of IgA-EmA and AGA paralleled the severity of the jejunal changes in patients with coeliac disease.

Granular IgA deposits in the dermal papillae are the hallmark of dermatitis herpetiformis (DH). They are associated, as a rule, with gluten-sensitive enteropathy (Katz & Strober, 1978; Fry, 1979).

Previous studies showed that antibodies of the IgA class to the smooth muscle endomysium of the gastro-intestinal tract (IgA-EmA) as well as IgG class anti-gliadin antibodies (AGA) can be detected by indirect immunofluorescence (IF) on sections of monkey oesophagus in a high proportion of sera of patients with DH and coeliac disease (Chorzelski, 1983; Chorzelski *et al.*, 1983). Our present studies which extend these previous findings reveal a quantitative relationship between the severity of the jejunal enteropathy and the titres of both IgA-EmA and AGA.

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METHODS

Patients

We studied thirty-eight patients with DH with granular papillary or junctional IgA deposits in the skin, twenty-seven patients with confirmed coeliac disease, nine cases with other enteropathies,* fifteen cases with ulcerative colitis, fifteen patients with linear IgA bullous dermatosis (LABD), five cases of pemphigus, six patients with bullous pemphigoid, forty with other skin conditions, and forty-one with systemic disease [thirty-four with systemic scleroderma and seven with systemic lupus (SLE)]. All cases of DH were confirmed by the direct IF demonstration of granular or fibrillar IgA deposits in the dermal papillae or at the dermo-epidermal junction. The evaluations of the jejunal mucosae were performed by the Paediatric Gastroenterology Department of the Warsaw Medical School. Standard criteria for the diagnosis of coeliac disease were employed (Townley, Khaw & Shwachman, 1965).

Immunofluorescence methods

Indirect immunofluorescence tests were performed primarily on oesophagi of selected species using human sera and anti-human IgA or anti-human IgG conjugates for both the IgA-EmA and AGA tests using the reagents described below according to the following format (Chorzelski *et al.*, 1983; Beutner, Nisengard & Kumar, 1979).

Pretreatment	Sera	Conjugate	Test for
None	Yes	anti-IgA	IgA-EmA
None	Yes	anti-IgG	(control)
Gliadin 0·1%†	Yes	anti-IgG	AGA

More specifically, comparative studies were performed on monkey jejunum and the gastro-intestinal tract of rat, mouse, cattle, goat and guinea-pig. The characteristics of the conjugates (Beutner *et al.*, 1979) were as follows: anti-IgG (Hyland) molar fluorescein to protein (F/P) ratio

TABLE 1. Species specificity of IgA anti-endomysium antibodies and anti-gliadin antibodies

	IgA class anti-endomysium antibodies				Sera positive on all substrates
	Monkey oesophagus	Cattle oesophagus	Calf oesophagus	Goat oesophagus	
Sera positive/sera tested	6/6	4/6	4/6	3/6	3/6
Sera positive for AGA/sera tested	11/11	8/11	8/11	10/11	8/11
Examples of EmA titres	80	10	neg.	neg.	
	160	40	40	10	
	160	20	20	10	
Examples of AGA titres	160	320	640	320	
	160	160	80	320	
	40	20	10	20	

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* The other enteropathies included the following: one case enteropathy responding to gluten-free diet and urinary infection; one case malabsorption; three cases milk (sensitive) enteropathy; two cases chronic diarrhoea; one case genetic defect of saccharidase; one case inborn IgA deficiency with malabsorption.

† Gliadin: Catalog No. G3375, Sigma Chemical Co., P.O. Box 14508, St Louis, Missouri 63178, U.S.A.

2·2, antibody protein 1·8 mg/ml, in a dilution of 1:32; anti-IgA (Hyland) molar F/P ratio 2·3, antibody protein 1·6 mg/ml, in a dilution of 1:16.

All serological studies were done blind, before data on the severity of the enteropathy were received.

RESULTS

Figure 1 depicts the indirect IF staining pattern of the recently described IgA-EmA (Chorzelski *et al.*, 1983) on the smooth muscle of monkey oesophagus. As can be seen in Figure 2, a different

TABLE 2. Frequency of IgA class anti-endomysial antibodies (IgA-EmA)[a] and IgG class anti-gliadin antibodies (AGA)[a] in dermatitis herpetiformis, coeliac disease and control sera

Clinical diagnoses	Type of Ab[a]	No. pos./no. tested	Antibody titre							
			Neg[b]	2·5	5	10	20	40	80	160
Dermatitis herpetiformis	IgA-EmA[a]	30/38 (78·9%)	8	6	2	7	7	4	4	1
	AGA[a]	24/38 (63·1%)	14	8[c]		6	6	2	1	1
Linear IgA bullous dermatosis	IgA-EmA	0/15 (0%)	15[d]							
	AGA	0/15 (0%)	15							
Coeliac disease	IgA-EmA	19/28 (67·9%)	9	1	6	3	4	3		2
	AGA	18/28 (64·3%)	10	3[c]			4	3	1	7
Other enteropathies	IgA-EmA	2/9 (22·2%)	7						2	
	AGA	4/9 (44·4%)	5	1[c]		1	1			1
Ulcerative colitis	IgA-EmA	0/15 (0%)	15							
	AGA	ND[e]								
Scleroderma and SLE	IgA-EmA	0/41 (0%)	41							
	AGA	0/13 (0%)	13							
Bullous and other skin diseases	IgA-EmA	0/51 (0%)	51							
	AGA	0/51	51							

[a] IgA-EmA = IgA class anti-endomysial antibodies to the reticulin-like structures of smooth muscle of the gastro-intestinal tract assayed by indirect IF staining on monkey oesophagus. AGA = anti-gliadin antibodies of the IgG class assayed by indirect IF staining on gliadin-pretreated monkey oesophagus sections.

[b] Neg. = titre less than 2·5.

[c] Dilution greater than 1:2·5 not tested.

[d] An IgA class anti-BMZ antibody to a titre of 20 but no other significant IgA or IgG class antibodies.

[e] ND = not done.

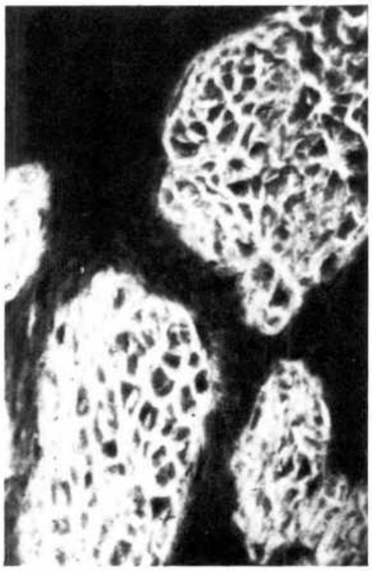


FIGURE 1

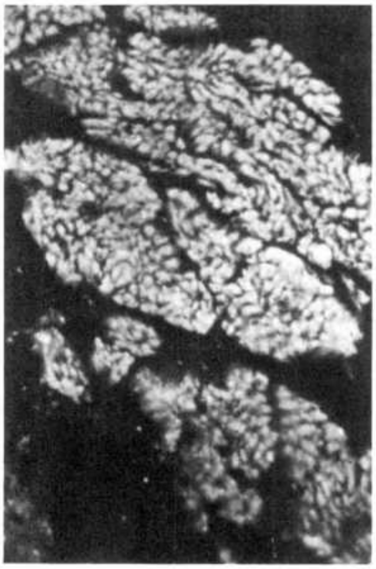


FIGURE 2

FIGURES 1 and 2. Indirect IF staining for IgA class antibodies to the smooth muscle layer of monkey oesophagus which is immediately distal to the luminal mucosa. Fig. 1 depicts the IF pattern of IgA-EmA. This stains the connective tissue structures which surround individual muscle fibrils (× 280). Fig. 2 illustrates the IF pattern of anti-smooth muscle antibodies of the IgA class. These antibodies react with the myofibrils giving a homogeneous fluorescence of the sarcoplasm (× 280).

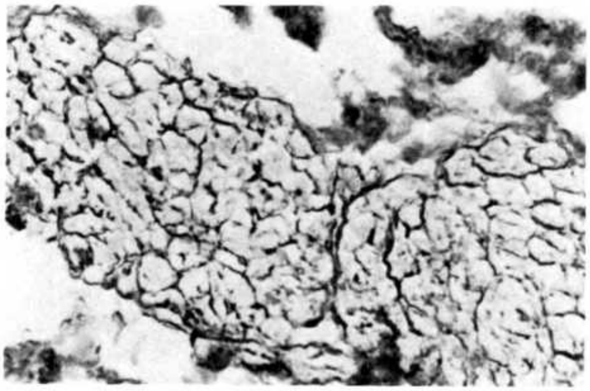
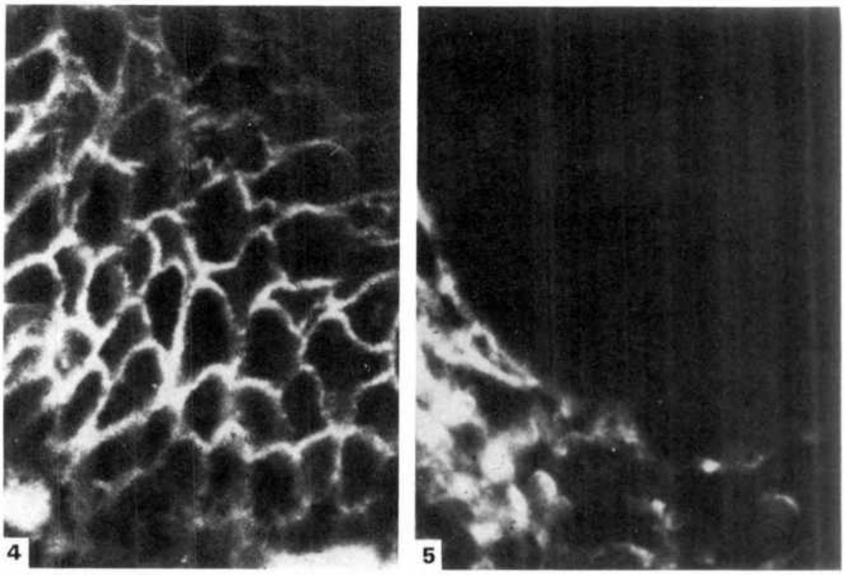


FIGURE 3. Belschowsky's silver impregnation staining of a section of monkey oesophagus smooth muscle layer. The structures stained appear to be comparable to those which react with IgA-EmA (× 280).

indirect IF pattern is obtained with serum containing the anti-smooth muscle antibodies associated with certain liver diseases (Johnson, Holborow & Glynn, 1965; Paronetto & Popper, 1976). The specific IF of the IgA-EmA appears in the connective tissue surrounding the myofibrils while the anti-smooth muscle antibodies stain the myofibrils homogeneously. Silver impregnation (Belschowsky's method) stains structures of smooth muscle (Fig. 3) which are



FIGURES 4 and 5. Mucosa of monkey oesophagus sections. Fig. 4. Mucosa of monkey oesophagus section pretreated with 0·1% gliadin in distilled water and then treated as for indirect IF staining using a 1:20 dilution of serum of a DH patient containing AGA at a titre of 40. Note the intercellular staining pattern which resembles that of pemphigus antibodies (×224). Fig. 5. Monkey oesophagus mucosa section treated as for Fig. 4 except that the pretreatment with gliadin was omitted. Note the negative IF reaction (×224).

similar to those reactive with the IgA-EmA, thus suggesting that the endomysial antigenic component may be associated with the intermyofibril substance of smooth muscle. Other organs, notably kidney and liver, do not react with IgA-EmA (Chorzelski *et al.*, 1983). These antibodies also do not react with endomysium of smooth muscle of the gastro-intestinal tract of mouse and rat. Monkey oesophagus seems to be superior to other substrates (Table 1).

Pretreatment of monkey oesophagus sections with gliadin followed by incubation with DH serum containing AGA and anti-IgG conjugate gives an IF pattern similar to that of pemphigus antibodies (Fig. 4). Negative IF staining (Fig. 5) obtained when gliadin is omitted proves that the pemphigus-like IF pattern depends on the presence of AGA in the patient's serum (Unsworth, *et al.*, 1981a). This control also serves to demonstrate that the EmA are predominantly or exclusively of the IgA class.

Table 2 summarizes the IF finding obtained with sera of patients with DH, LABD, coeliac disease and control sera from patients with other gut and skin diseases. As can be seen in Table 2, IgA-EmA was detectable in 79% of the DH cases but in none of the fifteen LABD sera examined. It is noteworthy that one of the fifteen LABD sera contained IgA class anti-basement membrane zone antibodies to a titre of 20. It is important that the IgA-EmA occurred not only in DH sera but also in about 68% of sera from coeliac disease cases. IgA-EmA also occurred in two of nine cases of other enteropathies examined, one in an enteropathy that responded to a gluten-free diet, and one in a patient with a saccharidase deficiency and gut changes of the type associated with coeliac disease. IgA-EmA were not detected in 107 other control sera examined. The AGA occurred with similar frequencies in DH and coeliac disease, with a lower frequency in other enteropathies but not in LABD or in other control sera (64 cases).

TABLE 3. Titres of IgA-EmA[a] and AGA[a] versus degree of gut villae flattening in coeliac disease

Type of antibodies	Severity of enteropathy	Neg. (<2·5)	2·5 or 5	10 or 20	40 or 80	160 or more	Totals
IgA-EmA[a]	Normal	3					3
	I/II	1					1
	II	4					4
	II/III	1	1				2
	III		1	4			5
	III/IV		3	1			4
	IV		2	2	3	2	9
	Totals	9	7	7	3	2	28
AGA[a]	Normal	3					3
	I/II		1				1
	II	2	1	1			4
	II/III	1	1				2
	III	1	1	1	1	1	5
	III/IV	1	1			1	3[b]
	IV	1	1	1	1	5	9
	Totals	9	6	3	2	7	27

[a] As for Table 1 footnotes.
[b] One not tested.

As shown in Table 3, further quantitative comparisons revealed a correlation between the titres of IgA-EmA and the degree of flattening of the villi of the jejunal mucosa in patients with coeliac disease and other enteropathies. The same holds true for AGA. It is evident from an examination of the data in Table 3 that the correlations between the severity of enteropathy and the tires of IgA-EmA as well as AGA were highly significant. Most patients with grades III, III/IV or IV enteropathy (14/19 cases) had both IgA-EmA and AGA. In all nineteen cases with such severity of enteropathy at least one of the two types of antibodies were present.

DISCUSSION

The IgA class antibodies to endomysium of the smooth muscle of the gastro-intestinal tract show a striking correlation to the severity of jejunal enteropathy as seen in these double-blind studies. This relationship is manifest not only by the correlation between the presence of the IgA-EmA and the more severe degrees of enteropathy (grades III, III/IV and IV), but also by the quantitative correlation of the titre of these antibodies to the degree of flattening of the gut mucosa. These findings indicate that detection and quantitation of IgA-EmA can be useful to gastroenterologists for studies of gluten-sensitive enteropathies and possibly also for other enteropathies associated with comparable flattening of the intestinal villae (Watt, Pincott & Harries, 1983). Such studies should be performed together with tests for AGA, at least until more experience on their correlation to IgA-EmA and the disease activity is gained. Recent studies on AGA suggest that the IgA class antibodies parallel disease activity more closely than do the IgG class AGA (Unsworth *et al.*, 1981b; Vainio *et al.*, 1983; Savilahti *et al.*, 1983). However, AGA of both IgG and IgA class, as detected by ELISA, was found in a comparable percentage in DH and pemphigoid, and in a proportion of control cases (Kieffer & Barnetson, 1983). Lane, Huff & Weston (1982) found a significant elevation of gluten in the bloodstream of patients with coeliac disease and DH, but about 5% of control sera had such elevated gluten levels, while only about 38% of sera from DH and 55% from coeliac disease had this antibody. Anti-reticulin IgA antibodies were found in DH in a low frequency (Seah *et al.*, 1973). The described IgA-EmA has not been found, so far, in any other diseases or controls.

The finding of IgA-EmA in 79% of the sera of DH patients in this study and in 68% of the DH sera in the initial study (Chorzelski *et al.*, 1983) raise questions about the possible role of these antibodies in the pathogenesis of the disease. It is well established that the severity of enteropathy in DH is lower or 'latent' (Weinstein, 1974) as compared with coeliac disease. Yet the frequency of IgA-EmA was found to be higher in the DH group than in coeliac disease in our studies. This may be due to the fact that the gluten-free diet is the primary mode of treatment of coeliac disease and that this treatment not only causes the normalization of the gut mucosa but also the disappearance of the IgA-EmA. The time for recovery of the gut mucosa is approximately 16 weeks (Weinstein, 1974). The time for disappearance of the IgA-EmA following the institution of a gluten-free diet remains to be determined. It is apparent that the dynamics of the formation and disappearance of the IgA-EmA must be studied. The capacity of the IgA-EmA to react with their homologous antigen in explants of normal gastro-intestinal tract mucosa has been studied since this aids in determining whether they are physiological or pathological autoantibodies (Beutner *et al.*, 1981 and unpublished data).

Regarding the relation of DH to LABD the studies provide further evidence that these are two distinct diseases with different aetiologies (Chorzelski, Jablonska & Beutner, 1979; Chorzelski *et al.*, 1981; Leonard *et al.*, 1983; Leonard *et al.*, 1982). The findings on the HLA types also support this (Lawley *et al.*, 1980). The relatively high concentrations of dimeric IgA in the granular deposits in DH skin (Leonard *et al.*, 1983) point to their origin in the gut, while the relatively higher concentrations of monomeric IgA in the linear deposits in the skin of LABD patients (Unsworth *et al.*, 1982) suggest that these deposits do not originate in the gut. The finding of IgA-EmA as well as AGA in both DH and in coeliac disease and other comparable gut diseases more clearly identifies DH as a distinct clinical manifestation of gluten sensitive enteropathy.

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ADDENDUM

After submission of this paper two of the nine cases listed as 'other enteropathies' in Table 2, positive for IgAEmA, proved to be coeliac disease. Thus, IgAEmA appears to be a specific marker of gluten-sensitive enteropathy.

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Effect of polyamine antimetabolites on cultured human keratinocytes from normal and uninvolved psoriatic skin

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SUMMARY

We describe the effect of two polyamine antimetabolites on polyamine and macromolecule synthesis of cultured human keratinocytes obtained by suction blisters from normal skin and the uninvolved skin of psoriatic patients. The concentrations of spermidine and spermine steadily increased during the culture of normal keratinocytes *in vitro*, whereas the putrescine concentration showed only a transient rise at the beginning of the active growth phase. Treatment with difluoromethylornithine decreased the concentrations of putrescine and spermidine in both normal and uninvolved psoriatic keratinocytes, but had no effect on either DNA or protein synthesis. Methylglyoxal bis(guanylhydrazone) marginally decreased the levels of spermidine and spermine and significantly inhibited the DNA and protein synthetic activities. Pretreatment of uninvolved psoriatic keratinocytes with difluoromethylornithine enhanced the accumulation of methylglyoxal bis(guanylhydrozone), resulting in a profound inhibition of cellular macromolecule synthesis. This synergistic effect was not seen in normal keratinocytes. Thus, although no statistically significant difference was observed between the cells derived from normal and uninvolved psoriatic epidermis, the psoriatic keratinocytes appeared to be more sensitive to the action of polyamine antimetabolites. The inhibition of DNA and protein synthesis by methylglyoxal bis(guanylhydrazone) was prevented by concomitant treatment with spermidine.

Polyamines play a role in cell proliferation, and inhibition of their synthesis prevents growth in many experimental systems (Jänne, Pösö & Raina, 1978). Clinical studies have shown that polyamine antimetabolites may provide a new tool for cancer therapy. Encouraging results have been recently obtained in the treatment of childhood leukaemia by the combination of two polyamine antimetabolites, difluoromethylornithine (DFMO), which is an irreversible inhibitor of ornithine decarboxylase (EC.4.1.1.17) and methylglyoxal bis(guanylhydrazone)

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(MGBG), a competitive inhibitor of adenosylmethionine decarboxylase (EC.4.1.1.50) (Siimes *et al.*, 1981). These two enzymes have an essential role in the regulation of polyamine biosynthesis. Ornithine decarboxylase catalyses the conversion of ornithine into putrescine, whereas the adenosylmethionine decarboxylase-catalysed reaction yields decarboxylated S-adenosylmethionine, a precursor of spermidine and spermine.

Epidermal hyperproliferation in psoriasis is accompanied by increased polyamine levels in the epidermis (Böhlen *et al.*, 1978; Lauharanta *et al.*, 1981; Lauharanta & Käpyaho, 1983), the serum and the urine (Russell & Durie, 1978; Proctor *et al.*, 1979). Polyamine concentrations are especially high in psoriatic lesions, but even uninvolved skin shows somewhat elevated levels (Böhlen *et al.*, 1978; Russell *et al.*, 1978). Recent studies have indicated that polyamine antimetabolites have a weak therapeutic effect when applied to psoriatic skin, accompanied by a decrease in the tissue polyamine levels (Voorhees, 1979; Grosshans *et al.*, 1980; Kousa *et al.*, 1982). Our recent studies on hyperproliferative mouse epidermis have indicated that 'priming' with DFMO before the application of MGBG results in a strong synergistic inhibitory effect on epidermal proliferation (Käpyaho, Lauharanta & Jänne, 1982; Käpyaho, Linnamaa & Jänne, 1982; Käpyaho, Lauharanta & Jänne, 1983). The combination of the two drugs could therefore also be effective in the treatment of psoriasis.

Proctor, Liu & Wilkinson (1980) reported earlier that DNA accumulation and protein synthesis were inhibited in cultured human foreskin keratinocytes in response to MGBG treatment. The present study deals with the effects of DFMO and MGBG alone and in combination on cultured human keratinocytes derived from both normal skin and uninvolved psoriatic skin.

METHODS

Keratinocyte cultures

The skin donors were healthy volunteers or patients with psoriasis at the Department of Dermatology, Helsinki University Central Hospital. The suction blisters were generated on healthy abdominal skin of each volunteer and on uninvolved abdominal skin of patients with psoriasis (thirty to thirty-six blisters of 5 mm diameter to each test person). The roofs of the blisters were cut off and trypsinized, and the dispersed keratinocytes were cultured in collagen-coated dishes in MCDB-151 medium (Peehl & Ham, 1980) supplemented with 10% pooled human AB serum (Finnish Red Cross Blood Service, Helsinki, Finland), hydrocortisone (10 μg/ml, Upjohn), penicillin and streptomycin as described earlier (Kariniemi *et al.*, 1982). The cells were incubated at 37°C in H_2O-saturated air containing 5% CO_2. Three to four dishes (6 cm in diameter) were prepared from each test subject.

In a few days keratinocytes proliferated to small cell colonies. The treatments were started after 10 days' culture, when the cells covered half of the bottom of the dishes. At the beginning of the treatments, the medium was changed to MCDB-151 lacking putrescine. However, in experiments with MGBG and spermidine (Table 4) the original medium was maintained throughout the experiment. DFMO (2 mM final concentration) was added to the respective cultures. MGBG (2 μM) and spermidine (2 μM) were supplied 4 days later, and the treatments were continued for three more days. The media were changed every 2 days. After the 7 days' treatment the cells were transferred into a medium containing no non-radioactive thymidine and incubated in the presence of [^{3}H]thymidine (10 μCi/ml for 60 minutes) and [^{14}C]leucine (1 μCi/ml for 45 minutes). After the labelling period, the cultures were washed three times with phosphate-buffered saline, trypsinized and pelleted by centrifugation. The cell pellets were suspended in 0·2 M perchloric acid.

Chemicals

DFMO was a gift from the Centre de Recherche Merrell International (Strasbourg, France). MGBG was synthesized by Orion Pharmaceutical Company (Helsinki, Finland). 6-[^{3}H]Thymidine (sp. act. 30 Ci/mmol) and L-U-[^{14}C]leucine (sp. act. 342 mCi/mmol) were purchased from Amersham International (Amersham, U.K.).

Analytical methods

Polyamines were determined from perchloric-acid extracts by the methods of Seiler (1970) as modified by Dreyfuss *et al.* (1973). MGBG content was determined enzymically from neutralized perchloric-acid extracts by the method of Seppänen *et al.* (1980). The perchloric-acid precipitates contained both the cellular DNA and protein. DNA was solubilized by hydrolysis in 5% trichloroacetic acid (30 minutes at 90°C). The incorporation of radioactive thymidine into DNA was determined from the solution by liquid scintillation counting, and the amount of DNA was assayed by the method of Giles & Myers (1965). The remaining protein precipitate was washed, dissolved in 0·1 M NaOH, and counted for radioactivity. Quantitation of the protein was performed by the method of Lowry *et al.* (1951).

Statistical analyses

Student's *t*-test for dependent variables was used for statistical analyses. The culture treated with DFMO, MGBG or both was always compared with the respective control culture.

RESULTS

Growth and polyamine levels of human keratinocytes in culture

About 50–80% of the keratinocytes adhered to culture dishes after inoculation, which resulted in an initial decrease in the amount of DNA per dish (Fig. 1A). The protein concentration of the cells rose steadily during the culture (Fig. 1A), obviously indicating that synthesis of prekeratin

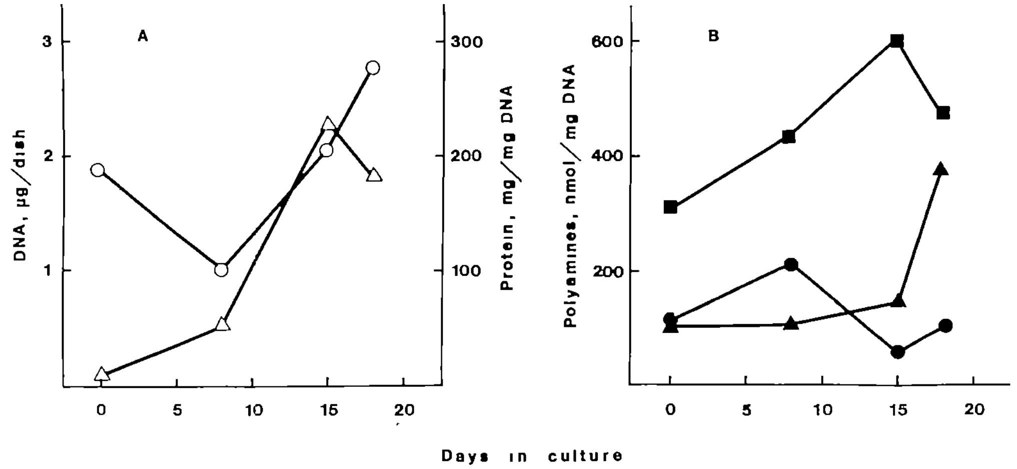

FIGURE 1. DNA, protein (A) and polyamine (B) levels of human keratinocytes during culture. (○——○) DNA; (△——△) protein; (●——●) putrescine; (▲——▲) spermidine; (■——■) spermine. Each point represents the mean of two separate cultures.

proteins took place in the differentiating cells. The concentration of putrescine rose after 8 days in culture (Fig. 1B). Thereafter it declined. On the other hand, the concentrations of spermidine and spermine increased during the active growth phase (Fig. 1B). In agreement with earlier observations (Procter *et al.*, 1980) the concentration of spermine in the cultured keratinocytes was high as compared with that of spermidine.

Effect of DFMO and MGBM on polyamine levels and macromolecule synthesis in cultured keratinocytes

Table 1 shows that after the culture period of 17–21 days, the polyamine levels in uninvolved psoriatic keratinocytes were considerably higher than those in normal keratinocytes, although the scatter between the individual cultures was wide. Treatment of the cultures with 2 mM DFMO for 7 days resulted in a decrease in cellular putrescine and spermidine levels (Table 1). The small decrease in the putrescine level was probably due to the fact that the serum used in the growth medium possibly contained small amounts of the diamine. DFMO treatment had practically no effect on either DNA (Table 2) or protein (Table 3) synthesis. The effects of 2 μM MGBG on spermidine and spermine levels after 3 days' treatment were marginal, but there was a highly significant rise in the putrescine concentration of the uninvolved psoriatic keratinocytes (Table 1) indicating that MGBG blocked the conversion of putrescine into spermidine. Since the intracellular concentration of MGBG was the same both in normal and in uninvolved psoriatic keratinocytes, the lack of putrescine accumulation in normal keratinocytes may reflect the slower rate of putrescine synthesis in these cells. MGBG treatment caused a dramatic inhibition of DNA synthesis both in normal and in uninvolved psoriatic keratinocytes (Table 2), and the synthesis of cellular proteins was also significantly inhibited (Table 3).

The effect of DFMO 'priming' was tested by combining the two treatments. The cells were

TABLE 1. Effect of difluoromethylornithine (DFMO), methylglyoxal bis(guanylhydrazone) (MGBG) and their combination on putrescine (Put), spermidine (Spd), spermine (Spm) and MGBG concentrations (all expressed as nmol/mg DNA) in keratinocytes. The cells were cultured for 10–14 days, and then they were transferred into medium without putrescine, and DFMO (2 mM) was added. MGBG (2 μm) was supplied 4 days later, and the culture was continued for 3 more days. After the 7 days' treatment with the polyamine antimetabolites, the cells were harvested and analysed as described in Methods. All values represent the mean ± s.e.m. of cultures from four different donors.

Cells and treatment	Put	Spd	Spm	MGBG
Normal keratinocytes				
None	31 ± 15	77 ± 22	173 ± 27	
DFMO	22 ± 6	32 ± 9	224 ± 74	
MGBG	30 ± 8	54 ± 16	155 ± 50	258 ± 39
DFMO + MGBG	38 ± 2	77 ± 15	278 ± 57	214 ± 41
Uninvolved psoriatic keratinocytes				
None	94 ± 54	594 ± 396	1288 ± 422	
DFMO	47 ± 24	153 ± 69	838 ± 297	
MGBG	214 ± 67***	329 ± 123	947 ± 333*	243 ± 69
DFMO + MGBG	36 ± 4	181 ± 85	1028 ± 400	342 ± 76***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 2. Effect of difluoromethylornithine (DFMO), methylglyoxal bis(guanylhydrazone) (MGBG) and their combination on the incorporation of radioactive thymidine into DNA (c.p.m./μg DNA), and on DNA content (μg DNA/dish) of keratinocyte cultures derived from either normal or uninvolved psoriatic skin. Experimental details are as in Table 1. Before collection, the cells were labelled with [^{3}H]thymidine for 60 minutes

	[^{3}H]Thymidine incorporation		DNA content	
Treatment	Normal	Uninvolved psoriatic	Normal	Uninvolved psoriatic
None	2491 ± 522	4197 ± 757	13·9 ± 0·3	10·1 ± 0·6
DFMO	3087 ± 577	3184 ± 720	13·1 ± 1·3	11·7 ± 1·0
MGBG	818 ± 224*	1013 ± 646***	11·0 ± 1·3*	10·0 ± 0·5
DFMO + MGBG	1105 ± 312*	685 ± 395***	10·3 ± 0·7*	8·9 ± 1·3

*$P < 0{\cdot}05$; ***$P < 0{\cdot}001$.

TABLE 3. Effect of difluoromethylornithine (DFMO), methylglyoxal bis(guanylhydrazone) (MGBG) and their combination on the incorporation of radioactive leucine into protein (c.p.m./μg protein), and on protein content (μg/dish) of keratinocyte cultures derived from either normal or uninvolved psoriatic skin. Experimental details are as in Table 1. Before collection, the cells were labelled with [^{14}C]leucine for 45 minutes

	[^{14}C]Leucine incorporation		Protein content	
Treatment	Normal	Uninvolved psoriatic	Normal	Uninvolved psoriatic
None	14·3 ± 1·7	13·7 ± 0·5	949 ± 8	905 ± 102
DFMO	14·3 ± 0·7	12·4 ± 1·3	778 ± 28	860 ± 87
MGBG	9·1 ± 0·7*	8·3 ± 0·8***	698 ± 101*	753 ± 79**
DFMO + MGBG	9·8 ± 0·6	7·6 ± 1·1***	671 ± 75*	726 ± 96**

*$P < 0{\cdot}05$; **$P < 0{\cdot}01$; ***$P < 0{\cdot}001$.

first grown in the presence of DFMO alone for 4 days, and then MGBG was added for 3 additional days. Table 1 shows that the treatment had no additive effect on the polyamine levels of the keratinocytes. However, the polyamine depletion caused by DFMO pretreatment significantly stimulated the accumulation of MGBG in uninvolved psoriatic cells, whereas no effect was observed in normal keratinocytes (Table 1). Interestingly, a similar difference existed between DNA and protein synthetic activities (Tables 2 and 3, respectively). While DFMO pretreatment caused no additional inhibition, in comparison with MGBG alone, of either macromolecule synthesis in normal keratinocytes, the inhibition by MGBG in uninvolved psoriatic cells was enhanced by the prior exposure of the cells to DFMO.

Tables 2 and 3 indicate that MGBG decreased the amount of cellular protein more efficiently than the amount of DNA, which probably results from the slower rate of DNA synthesis in the cultures.

Reversal of the effect of MGBG by spermidine

MGBG is an analogue of spermidine, and competes with the polyamine for membrane transport system and intracellular binding (Dave & Caballes, 1973; Seppänen, Alhonen-Hongisto & Jänne, 1981). When 2 M spermidine was added to cultures of uninvolved psoriatic keratinocytes together with 2 M MGBG, the former, apparently having a higher affinity to the physiological binding sites, prevented the uptake and cellular accumulation of MGBG (Table 4). Thus spermidine treatment totally abolished any inhibition of DNA and protein synthesis by MGBG. A similar effect was observed in normal keratinocytes (results not shown).

TABLE 4. Reversal by spermidine of the effect of methylglyoxal bis(guanylhydrazone) (MGBG). The cells were cultured in putrescine-containing MCDB-151 medium for 14–18 days, whereafter MGBG (2 μM) and spermidine (2 μM) were added. Three days later, the cultures were labelled with [^{3}H]thymidine (for 60 minutes) and [^{14}C]leucine (for 45 minutes), harvested and analysed as described in Methods. The cellular concentration of MGBG is expressed as nmol/mg DNA. The results represent the mean ± s.e.m. of five cultures derived from the uninvolved skin of psoriatic patients

Treatment	[^{3}H]Thymidine incorporation	Leucine incorporation	MGBG
None	3268 ± 472	12·0 ± 1·9	—
MGBG	961 ± 466**	9·7 ± 1·9*	249 ± 103
MGBG + spermidine	2930 ± 1018	12·3 ± 3·5	33 ± 22*

$^*P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$.

DISCUSSION

Cultured human keratinocytes obtained from suction blister roofs are not contaminated by dermal fibroblasts (Kariniemi *et al.*, 1982) and are therefore ideal target cells for studies on the effects of polyamine antimetabolites on human keratinocytes under defined conditions. Polyamine levels of normal keratinocytes increase during the culture, which may indicate active cell proliferation (Fig. 1B). On the other hand, a portion of the cells continuously stops dividing and differentiates gradually. The differentiation is accompanied by rapid accumulation of cellular proteins (Fig. 1A), and upon reaching confluency, half of the cells are partially or fully keratinized (Kariniemi *et al.*, 1982).

High polyamine levels are characteristic of both psoriatic lesions and uninvolved psoriatic epidermis (Böhlen *et al.*, 1978; Lauharanta *et al.*, 1981). Interestingly, the polyamine concentrations of the uninvolved psoriatic keratinocytes in culture were increased compared with normal keratinocytes (Table 1), although there was considerable variation between the individual samples. The DNA synthetic activity of the uninvolved psoriatic cells also seemed to be somewhat higher than that of the normal cells (Table 2). This is in accordance with the earlier results of Harper, Rispler & Urbanek (1978) who have shown that the labelling index of epidermal explants from both involved and uninvolved psoriatic skin became equal, and considerably higher than that of normal skin, after an 8-day culture *in vitro*. In addition, the psoriatic keratinocytes both from the lesion and from uninvolved areas show an abnormally high proliferation rate after grafting to nude mice (Krueger, Chambers & Shelby, 1981; Briggman & Lazarus, 1982). Further, both lesional and uninvolved keratinocytes appear to have altered cell surface properties compared with normal keratinocytes (Gommans *et al.*, 1982; Kariniemi, Lehto & Virtanen, 1983; Kariniemi *et al.*, 1983). All these findings suggest an altered structure of psoriatic keratinocytes being expressed in both lesional and uninvolved skin.

Studies *in vivo* with hyperproliferative mouse epidermis have shown that topical MGBG penetrates mouse skin (McCullough *et al.*, 1983), and either alone or especially in combination with DFMO, profoundly inhibits the synthesis of both DNA and proteins (Käpyaho *et al.*, 1983). The results in Tables 2 and 3 indicate that MGBG is also inhibitory in human keratinocytes. The effect of DFMO 'priming' appears to be only moderate in comparison with the mouse epidermis model, in which DFMO pretreatment produced a several-fold increase of the tissue MGBG concentration (Käpyaho *et al.*, 1982) and considerably enhanced its antiproliferative activity (Käpyaho *et al.*, 1983). An additive effect could, however, be observed in human cultures derived from uninvolved psoriatic skin (Tables 1 and 2). The resistance of human keratinocytes to DFMO 'priming' is probably due to their relatively slow rate of proliferation. The turnover of polyamines is slow in such conditions, and prolonged treatment with the antimetabolites is required to achieve an effective polyamine depletion. Also, since the uptake of MGBG is directly proportional to both the rate of cell division and the extent of polyamine depletion, high intracellular MGBG concentrations are not easily achieved in conditions of slow proliferation. However, our results suggest that the uninvolved psoriatic keratinocytes are more prone to the 'priming' effect of DFMO than the normal cells, apparently due to their somewhat higher rate of polyamine synthesis and cell proliferation *in vitro*. The suction blister technique does not allow the isolation of lesional psoriatic keratinocytes, but DFMO 'priming' might result in considerable synergistic effect in these cells. *In vivo* the rapidly proliferating lesional epidermis would probably be more sensitive to 'priming' than the surrounding uninvolved epidermis, resulting in a selective effect against the areas involved with psoriasis.

The effect of MGBG can be totally prevented by concomitant treatment of the cells with equimolar concentrations of spermidine (Table 4). Spermidine inhibits both the uptake, and probably also the intracellular binding of MGBG, and it could be used in connection with MGBG treatment to alleviate drug toxicity, for the growth-inhibitory effects of MGBG in keratinocytes are reversible for several days (Proctor *et al.*, 1980). In therapeutic use, this could provide a valuable means of avoiding skin irritation produced by an extensive accumulation of MGBG in the tissue (Weinstein *et al.*, 1981).

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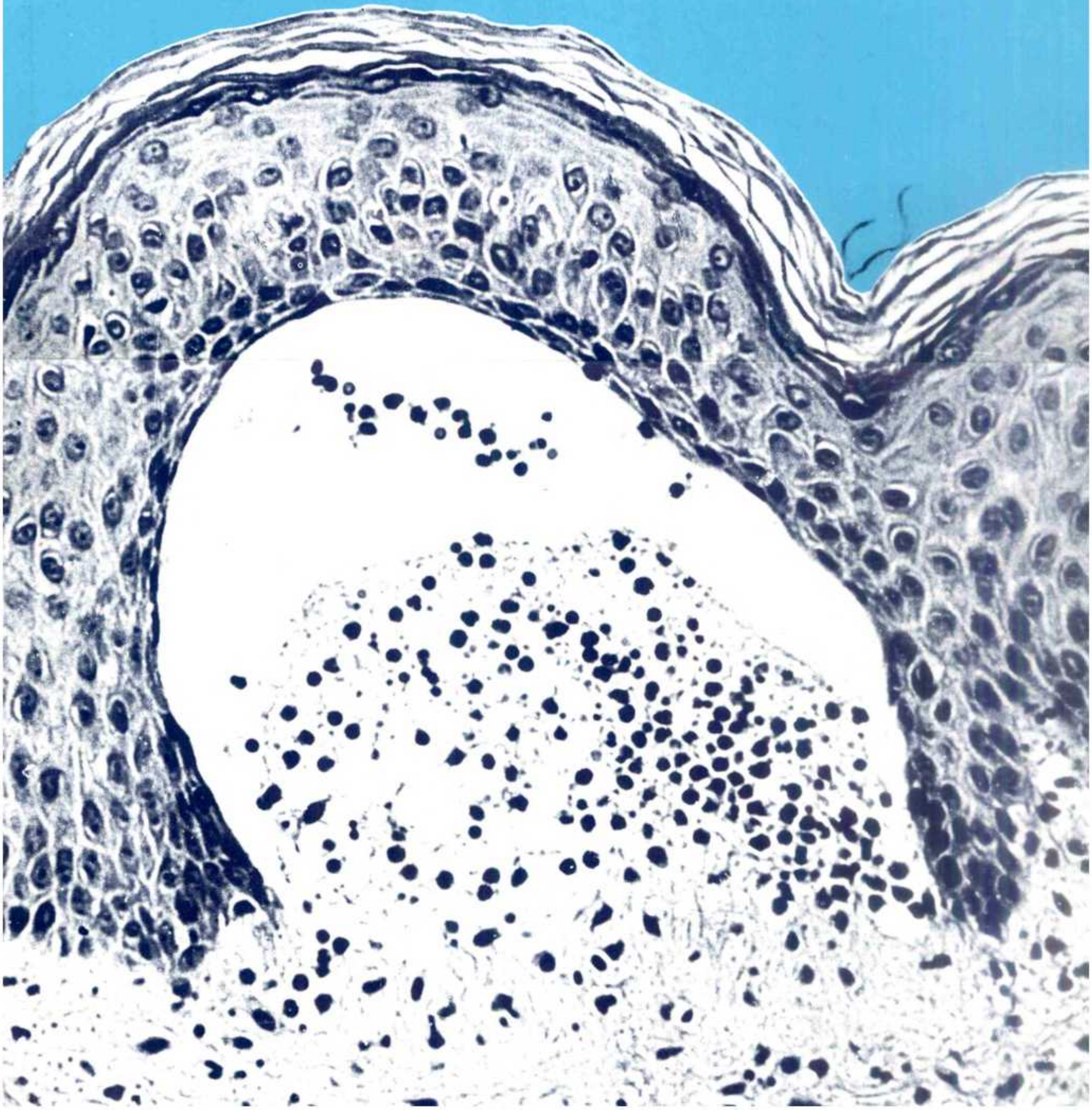

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This book is an extensive revision of 'An Introduction to the Diagnostic Histopathology of the Skin' by the late Professor Milne. This new edition, based on plans left by Professor Milne, has been edited and revised by his Glasgow colleagues led by Professor Rona MacKie, thus ensuring that the approach to dermatopathological problems used in the first edition is continued in the second.

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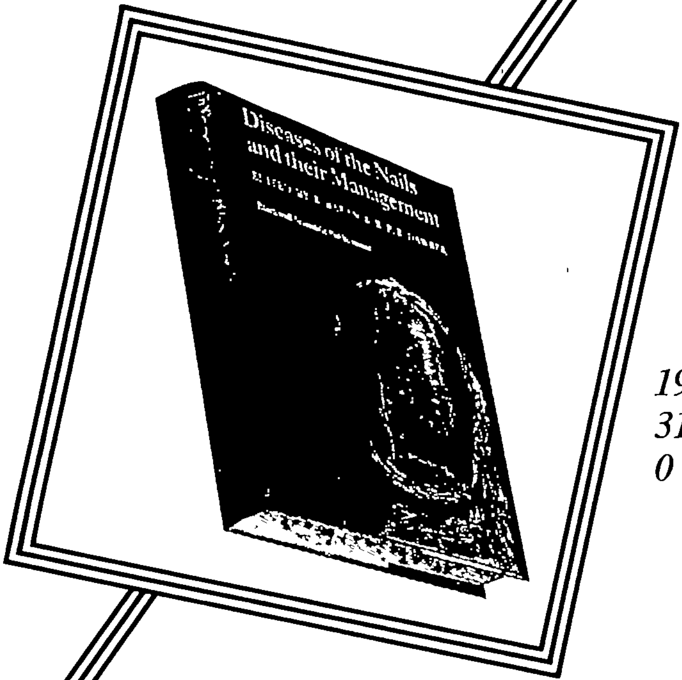

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Porphyria turcica due to hexachlorobenzene: a 20 to 30 year follow-up study on 204 patients

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SUMMARY

During 1955–1961 in south-east Turkey, over 3000 patients developed porphyria due to ingestion of hexachlorobenzene, a fungicide added to wheat seedlings. Subsequently they developed pigmentation, hirsutism, weakness, porphyrinuria and bullae. The condition was called *kara yara* or 'black sore'. Many of the breast-fed children under the age of 1 year died from a disease known as *pembe yara* or 'pink sore'.

In this follow-up study of 204 patients, 20–30 years later, there were 132 males and 72 females, average age 32·1 years (at time of examination), with an average age of onset of 10·2 years, and duration of 2·4 years. Neurological, dermatological, and orthopaedic abnormalities still persisted. Neurological symptoms included weakness (66%), paraesthesiae (54%), neuritis (63%), myotonia (49%), and occasional 'cogwheeling' (29%). Some individuals affected before puberty had small stature (44%), small hands (64%) and painless arthritis (67%). Severe residual scarring was common (85%), and pinched facies (42%), hirsutism (47%) and hyperpigmentation (71%) were also observed. Enlarged thyroids were observed in 37% (59% in females), which was above the average of 5% in this area of Turkey. Porphyrin excretion, particularly uroporphyrin in the urine and stools, was still significantly increased in seventeen patients. Hexachlorobenzene levels as high as 2·8 p.p.m. were detected in human milk, and the average level (0·29 p.p.m. ± s.d. 0·06) was 140 times the level allowed in cow's milk.

Sporadic cases of porphyria in eastern Turkey first occurred in 1955 and became more common until 1958 when the probable relationship to a fungicide, hexachlorobenzene (HCB), became known (Cam, 1959). New cases became less frequent after 1960 (Dogramaci, 1962a; Cam, 1963). In 1957 Cam, a dermatologist in Diyarbakir, described the first cases as resembling congenital erythropoietic porphyria, but soon realized that the cause of the porphyria was acquired. The commercial fungicides, Chlorable, Suresan A, Amatin and Leytosan, containing 20% HCB, were imported from western Europe in 1954 for mixing with seed wheat, to control a wheat fungus, *Tilletia tritici*. The proportion of commercial fungicides containing HCB was 2 kg to

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1000 kg of wheat. The arrival of this recently shipped seed wheat to eastern Turkey had been delayed past planting time and was unfortunately used to replace the edible stores of wheat, as bread or bulgar, which was a common practice in times of scarcity.

It was estimated that between three and four thousand Turks developed porphyria of varying severity (Dogramaci, 1962a) a condition which was not known to have occurred in the preceding generation of Turks. Porphyria turcica was subsequently observed in several genetically distinct populations such as old Turkish settlers, immigrant Turks and Kurds (Dogramaci et al. 1962) suggesting no genetic predisposition to the development of porphyria. Subsequent experimental studies by numerous investigators (Schmid, 1960; Kantemir, Giner & Kayaalp, 1960; Ockner & Schmid, 1961; De Matteis, Prior & Rimington, 1961; Pearson & Malkinson, 1965) have shown that HCB (C_6Cl_6) will produce hepatic porphyria in treated animals.

Distribution of hexachlorobenzene-treated wheat

Original Turkish government records describing areas of distribution of the treated wheat have been obtained. The report indicates that the seeds treated with HCB were distributed between 1954 and 1959, mainly to south-eastern Anatolia (Guneydogu and Anadolu), including the cities of Diyarbakir, Urfa, Siirt, Mardin, Gazientep, Mus, Elazig, Nigde, and Adapazari.

Clinical features of porphyria turcica in 1955–1961

The interval between HCB ingestion and the development of symptoms was calculated to be approximately 6 months. The initial symptoms mentioned by most subjects were weakness, loss of appetite, and sunlight sensitivity. Hyperpigmentation was maximum in exposed areas of skin, and hypertrichosis occurred primarily on the forehead, cheeks, arms and legs, and was

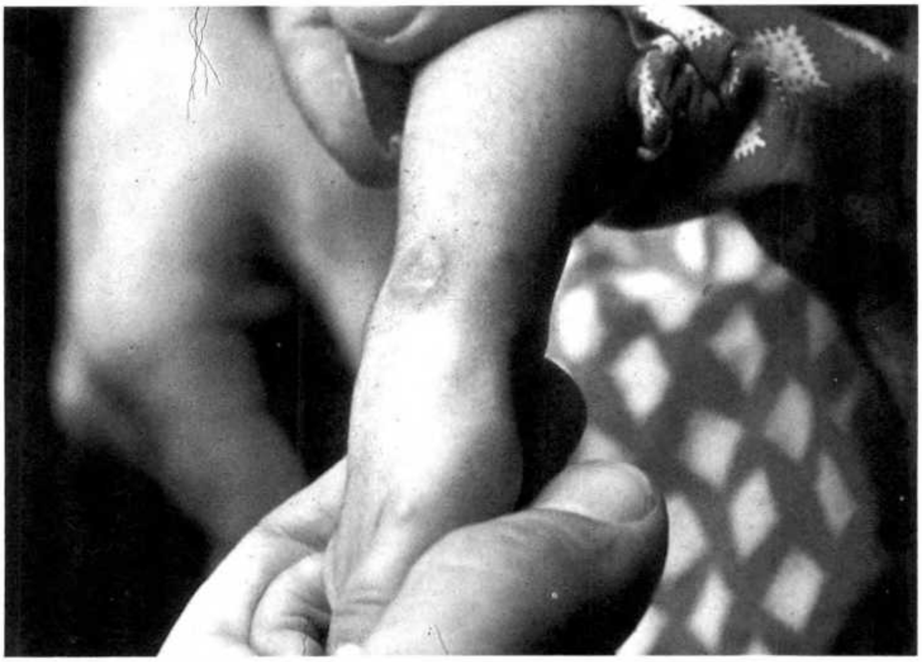

FIGURE 1. Illustrates the 'pink sore' or *pembe yara* on the dorsum of the right ankle in a breast-fed child (1961).

sufficiently distinctive in the 5–15 year age group for them to be described as 'monkey children'. Development of bullae up to 5 cm in size occurred frequently, healing with severe mutilating scars. This porphyria was known as *kara yara* ('black sore'). During the early period of active porphyria the affected persons continued to be irritable with colic, loss of appetite, weakness, and excretion of red or brown urine (porphyrinuria). These acute symptoms became less frequent after 5 years. Arthritis was common in the younger age group with swelling and spindling of the fingers but very little pain.

Accurate records are not available, but it has been estimated that between 1000 and 2000 children died during the first year of life from a condition known as *pembe yara* or 'pink sore' (Fig. 1), in which many children breast-fed from affected mothers died with associated weakness, convulsions, and annular erythema (Kantemir, Cam & Kayaalp, 1960; Cripps *et al.*, 1981). It became apparent during our visits to the Turkish villages that cases of *pembe yara* occurred more frequently after the major outbreak of *kara yara* or porphyria, mainly between 1959 and 1962.

Dogramaci (1962a) in an extensive survey of 592 patients in 1964 reported that the incidence of porphyria was slightly greater in males (381) than females (211). Most patients were between the ages of 6 and 15 years (83%), whereas the incidence in adults over the age of 20 years was considerably less (6%). The essential clinical features observed in a report on 547 patients from age 3 to 20 years were bullae (99·6%), porphyrinuria (95·4%), weakness (94·7%), hirsutism (82·3%), hyperpigmentation (64·9%), and arthritic changes (54·8%). Hepatomegaly was found in most patients early in the disease but became less noticeable in the less active period.

Porphyria turcica in 1977–1983

The purpose of this study is to describe the clinical features of porphyria turcica 20–30 years after the onset. The investigators have made eight field visits into eastern Turkey, primarily around the town of Diyarbakir and the surrounding villages within a diameter of approximately 500 km. A preliminary study of thirty-two patients was reported by Cripps, Peters & Gocmen (1979).

METHODS

Two hundred and four Turkish patients (132 males) with a past history and recognizable features of porphyria were studied. Urine and stool specimens for porphyrin determinations and porphyrin precursors were obtained from these patients as well as from controls; these latter were selected from those who were born either after 1961 or who had not lived in the affected area in the past 5 years and who had no history of porphyria.

Tissue samples for hexachlorobenzene (HCB) assay were obtained from porphyric and control subjects, consisting of maternal milk, blood serum, and subcutaneous fat. In addition, blood samples for a number of routine laboratory studies (essentially normal) were obtained. These are described in detail elsewhere (Peters *et al.*, 1982).

Methods of porphyrin determination

Faecal coproporphyrin, uroporphyrin, protoprophyrin, urinary coproporphyrin and uroporphyrin determinations, were carried out by method of Rimington (1961) and as described by Cripps and Peters (1967). Sodium bicarbonate and thymol were added as preservatives and specimens were refrigerated except for short periods during the 24 hours of air transport.

Porphyrin precursors, ALA and PBG, were determined by the method of Mauzerall and Granick (1956).

Methods of hexachlorobenzene (HCB) determination

Extracts of HCB from tissue in hexane were compared with a standard concentration of HCB using a Perkin-Elmer Sigma 4 gas chromatography and nickel 63 electron capture gas chromatography for serum, milk and fat (Thomas, 1974).

RESULTS

Clinical features 1977–1983

The average age of the 204 patients was 32·1 years (at time of examination), with the average age of onset of the disease 10·2 years, and mean duration 2·4 years.

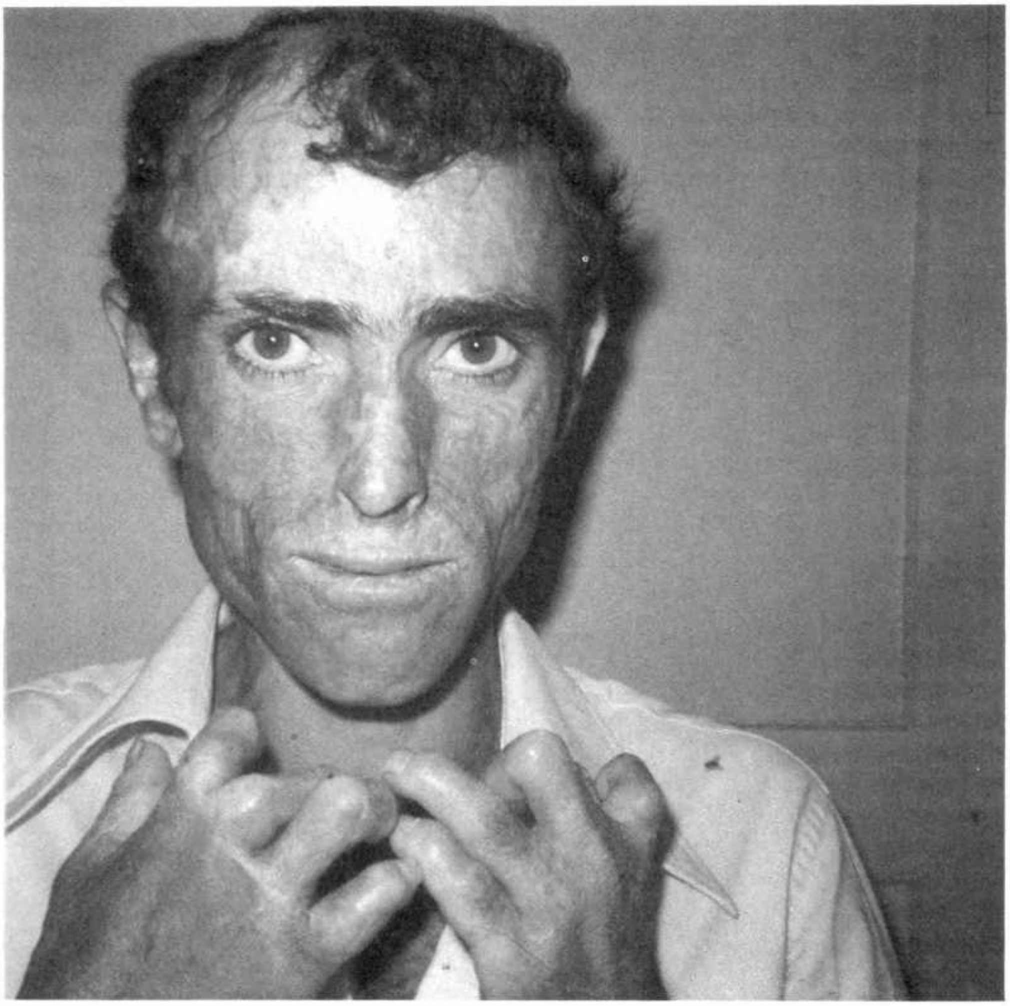

FIGURE 2. Severe scarring, pinched facies, hyperpigmentation, small hands and arthritis illustrated by case 82, male, aged 29 years (1979).

The majority of patients (174) were located in 12 villages surrounding the city of Diyarbakir.

Diyarbakir	27	Savur	7	Koprubasi	3
Bismil	47	Silvan	51	Tilalo	2
Hazro	21	Ergani	5	Sarabi	1
Simaki	16	Mazidagi	6	(Istanbul)	3
Bagivar	9	Derik	6		

The current health status of the hexachlorobenzene-exposed survivors and their children was evaluated by physical and clinical examination, routine haematology, and clinical chemistry, including T_3 and T_4 for thyroid function. Once patients had been identified they were subsequently followed for changes in clinical condition, including the development of any malignancy and congenital defects in their children.

Twenty to thirty years after the onset, the most common clinical features were those of chronic porphyria with hyperpigmentation (71·1%), large scars, usually on the cheeks, arms, and dorsa of the hands (85·6%), pinched facies (42·4%) (Figs 2 & 3), and hypertrichosis (47·5%). One of the most striking clinical features was a painless arthritis (67·1%) and small, distinctive hands (64·7%) (Fig. 4); sclerodermoid thickening of the skin on the fingers was surprisingly rare and not distinctive. The shape of the small hands with severe shortening of the digits was due to osteoporosis, particularly the terminal phalanx which was almost completely reabsorbed, which also occurred in other phalangeal, carpal, and metacarpal bones. Although there was arthritis and swelling of the joints, there was very little pain experienced by the patients and ulnar deviation, as observed in rheumatoid arthritis, was absent. Short stature

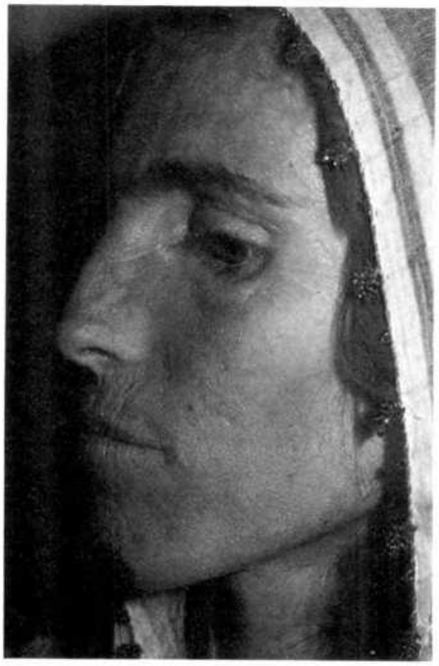

FIGURE 3. Pinched facies, fine scarring, patchy hyperpigmentation, hirsutism between eyebrows and scalp hair illustrated by case 154, female, aged 27 years (1981).

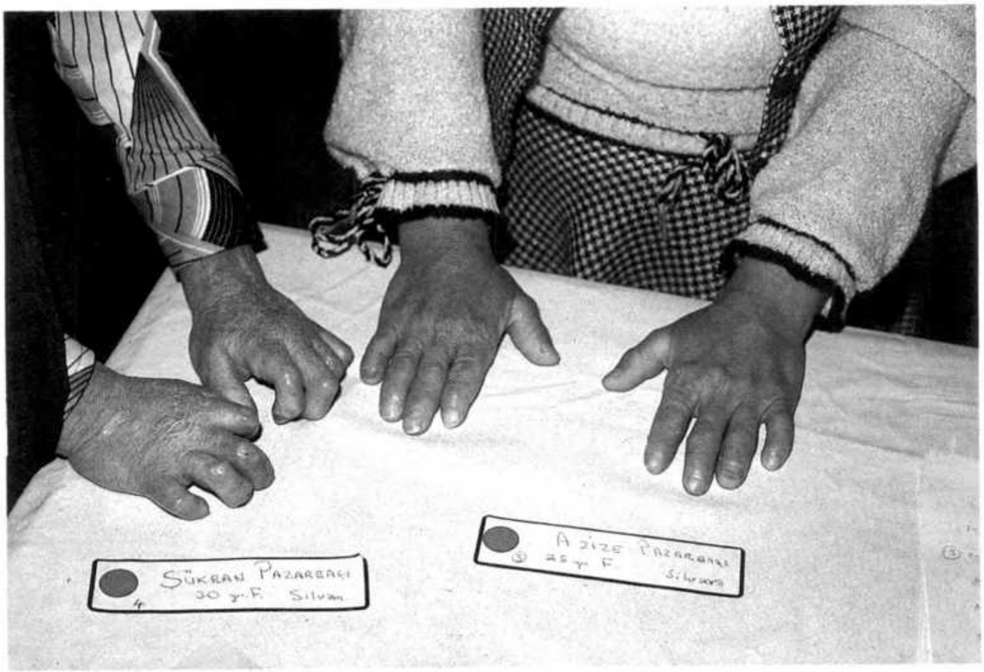

FIGURE 4. Arthritis and small hands with shortening of the digits, particularly the terminal phalanx, from severe osteoporosis shown in two sisters: (left) case 105, aged 30 years, and (right) case 106, aged 25 years, (1980).

occurred in 44·6%. An enlarged liver, a common finding in the early stage of this porphyria, was palpated in only nine subjects (4·4%). A clinically enlarged thyroid was detected in 76 subjects (37·3%), which was above the average of 5% in this 'goitre belt' of Southeast Turkey. Thyroid enlargement was predominant in women (43, 59·7%), in contrast to men (33, 25%). Weakness (66·1%), paraesthesia (54·9%), and neuritis with evidence of sensory shading (63·7%) was also detected. In addition to residual sensory neuropathy, there was also evidence of motor neuropathy suggested by a myotonic response to mechanical percussion in 59 of 119 patients (49.6%) and 'pseudo-cogwheeling' in 49 of 168 patients (29·2%). One patient had *grand mal* seizures, although this may be unrelated to HCB and porphyria. Patients needing hospital admission for conditions such as thyroid surgery are treated at Haceteppe Medical Center in Ankara, when additional studies such as nerve conduction, electromyography, and electroencephalography can be determined.

During the period of this study, two of the Turkish patients have died from liver failure: one a 27-year-old male with cirrhosis of the liver, and the other a 54-year-old female during treatment for pulmonary tuberculosis.

One hundred and eighty-eight total pregnancies were noted in forty-two women. There were fifteen fetal deaths, and thirty-one children died in the first few years after delivery, leaving 142 living children.

In recent years this area of south-eastern Turkey around Diyarbakir, located approximately 650 km south-east of Ankara, has gradually modernized so that many of the affected villagers have left the rural areas and are becoming harder to trace.

PORPHYRINS

A total of 204 urine specimens and 183 stool specimens from affected Turks had been tested for porphyrins by November 1983. The mean values are summarized in Table 1, with the range of values listed in parentheses, and compared with thirty-seven control Turks and forty U.S.A. controls (Cripps, 1974). ALA and PBG, porphyrin precursors, were obtained in 112 Turkish patients compared with controls in Table 2. Porphyrins in urine and stool from nine patients with the higher levels are shown individually and seventeen patients with the highest levels are included in a separate group. In this group the mean urine uroporphyrin of those patients was 106·4 μg/l (controls 5·66 μg/l), ranging as high as 1607 μg/l. Correspondingly in the stool the mean uroporphyrin was 24·4 μg/g dry wt, in contrast to the control mean of 1·41 μg/g dry wt, ranging as high as 189.2 μg/g dry wt. Porphyrin levels were only mildly elevated in the remaining 187 porphyric patients.

None of the affected patients had active disease, such as bullae, although 37% had a history of persistent mild fragility of light-exposed skin. There was no correlation between increased porphyrin levels and severity of clinical symptoms. The mean ALA of the 112 patients studied was 4·76 mg/l in contrast with the control level of 4·35 mg/l. Elevation of ALA in the urine in

TABLE 1. Porphyrins

	Stool (μg/g dry weight)			Urine (μg/l)	
	Copro-	Proto-	Uro-	Copro-	Uro-
HCB exposed					
Selected patients with highest porphyrins					
No. 10 (9/77)	154 9	6·1	189·2	779·3	1607 4
(6/78)	—	—	—	647·1	27·2
(9/78)	837·7	43·9	89 6	353·9	39·7
No. 12	4·4	17·7	2·2	202·5	34·8
No. 4	219·8	14·9	159·4	142 5	33·8
No. 17	1·0	2·4	1·7	342 0	50·2
No. 32	5·5	5·2	12·6	99·8	14·6
No. 41	3·0	0·7	0·7	45·1	63·2
No. 90	10·1	61·8	0·8	62·8	24·2
No. 185	4 9	12·0	4·0	169·4	31·0
No. 192	3·5	10 6	2·4	319 4	36·4
Highest porphyins (17)					
Mean	62·4	12·1	23·1	182·7	102 3
Range	(1 0–837·6)	(0 7–61·8)	(0·7–189·2)	32·6–779·3)	(16–1607)
Remainder (187)					
Mean	5·38	8·15	1·23	33·4	7·14
Range	(0·5–41)	(0–103·4)	(0–12·6)	(0–198·4)	(0–29·5)
Controls					
Turkey (37)					
Mean	4·80	7·65	1·41	32·7	5 66
s.d.	(3·2)	(9·83)	(1 57)	(23 6)	(4·25)
U.S.A. (40)					
Mean	6·1	21·1	2·8	69·0	9 0
s.d.	(4·7)	(11·6)	(2 7)	(27)	(4)

TABLE 2. ALA and PBG (mg/l)

	ALA	PBG
Turkish patients (112)		
Mean	4·76	0·45
Range	0·14–13·52	0·11–1·30
s.d.	2·38	0·23
Turkish controls (26)		
Mean	4·35	0·40
Range	1·86–9·16	0·07–1·19
s.d.	1·72	0·26
U.S.A. controls (40)		
Mean	2·9	0·4
Range	1·1–4·9	0·1–1·0

both patients and Turkish controls was higher than U.S.A. controls. In two patients the ALA was 11·7 mg/l and 13·5 mg/l, but did not correlate with the symptoms. The porphobilinogen was normal. Although these porphyrin precursors may be increased in the urine in acute intermittent porphyria, there was no correlation of the higher ALA levels with the neurological symptoms in the Turkish group.

Hexachlorobenzene

Forty specimens of human milk from porphyric Turkish mothers were examined for quantitative HCB. The average value was 0·29 p.p.m. (s.d. 0·06); the highest level in one patient 2·8 p.p.m., was observed 4 days post partum and decreased to 1 p.p.m. after 2 weeks' suckling. Forty-five specimens from controls and non-porphyric mothers from the same area showed a mean concentration of 0·22 p.p.m. (s.d. 0·28 p.p.m.). Further control specimens from outside this area of south-east Turkey are currently being obtained.

COMMENT

Bleiberg *et al.* (1964) described acquired porphyria in factory workers from New Jersey following exposure to 2,4-dichlorophenoxy acetic acid (2,4-D) and 2,4,5-trichlorophenoxy acetic acid (2,4,5-T), both of which resemble HCB. Symptoms disappeared after cessation of exposure. In our HCB study neurological, determatological and orthopaedic abnormalities have persisted. An enlarged thyroid was observed in 60% of female patients, well above the average of 5% in this area of south-east Turkey. Cabral *et al.* (1977) reported that HCB fed to hamsters caused thyroid neoplasia, but a thyroidectomy on one patient, a 30-year-old female from Derik, showed that the thyroid enlargement was not neoplastic and the histology was compatible with benign goitre. The thyroid, however, began to enlarge again within 18 months.

Porphyrin excretion, in the urine and/or the stool, particularly uroporphyrin, was significantly increased in seventeen patients (Table 1).

The question might be raised whether these patients with increased porphyrins have been subsequently exposed to other porphyrinogenic agents such as alcohol. Most patients did not consume alcohol for religious reasons, and several males that consumed above-average amounts of alcohol did not have any significant increase of porphyrins. Results of animal experiments with HCB confirm that the chemical is toxic and porphyrinogenic, with increased uroporphyrin in the urine and stool and a moderate increase in stool coproporphyrin, with a late increased production of urinary ALA and PBG. This pattern of increased urinary and stool uroporphyrin and coproporphyrin levels in porphyria turcica is similar to the porphyric animals (Ockner & Schmid, 1961; De Matteis *et al.*, 1961; Pearson & Malkinson, 1965). In the Turkish patients the porphobilinogen level was normal, although the mean ALA in 112 patients was 4·76 mg/l compared with controls of 4·35 mg/l. ALA was, however, increased in two patients, 11·7 mg/l and 13·5 mg/l (highest control was 9·16 mg/l). However, it is suspected that the neurological symptoms are related to neurotoxicity rather than to a form of acute intermittent porphyria (Laseter *et al.*, 1976; Courtney, 1979).

Peters *et al.* (1966) reported the presence of HCB in maternal milk in four mothers of *pembe yara* children. No quantitative data of early blood HCB levels in affected porphyric individuals or mothers of *pembe yara* children are available, but HCB is stored in body fat (Brady & Siyali, 1972). The mean HCB in maternal milk of porphyric mothers determined in forty specimens 20 to 25 years later was 0·229 p.p.m.; the highest was 2·8 p.p.m. observed 4 days post partum, which decreased to 1 p.p.m. after 2 weeks suckling. In an experimental study to simulate *pembe yara*, in which 0·2% HCB was fed for 1 week in mid-gestation to rats, it was found that the surviving litters had convulsions, weakness, and thrived poorly (Cripps *et al.*, 1981). Transplacental HCB absorption occurred with increased levels in the fetus in approximately 50% of the mothers. HCB levels in the maternal fat post partum was over 1200 p.p.m., but with prolonged administration levels over 5000 p.p.m. were observed. In another group, mother rats which had been suckling for 3 weeks, the quantity of HCB in the mammary gland decreased. Kroger (1972) noted that pesticides in human milk were higher in mothers nursing for the first time, whereas after three to four babies had been nursed, pesticide concentration fell below average.

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Transient and persistent photosensitivity due to musk ambrette. Clinical and photobiological studies

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SUMMARY

Musk ambrette caused contact photosensitivity in five patients. One patient showed transient light reactivity and four showed persistent light reactivity. Tests with artificial radiation were performed on normal skin without musk ambrette being added. All patients reacted abnormally to UV-B and three to UV-A. In particular the patient with transient light reactivity showed abnormal responses to UV-B and UV-A. The patients were not always aware of the role of sunlight in the development of their eruption and the clinical features were not always typical of a photodermatitis.

Allergic contact photosensitivity (CPS) is a form of delayed hypersensitivity that requires ultraviolet radiation (UVR) and a topically applied chemical for its induction. In the past the halogenated salicylanilides have been responsible for a large number of cases of CPS (Wilkinson, 1961). Some patients had transient photosensitivity which cleared when they avoided the chemical whilst others remained photosensitive even though they ceased all contact with the chemical. These latter patients sensitized to tetrachlorsalicylanilide (TCSA), were said to show 'persistent light reactivity' (PLR) (Wilkinson, 1962a). PLR has been seen after photosensitization with chemicals other than TCSA (Burry, 1967; Masuda *et al.*, 1971; Ramsay, 1979), and several of these compounds have now been removed from the market.

From time to time new chemicals that cause CPS and PLR are identified. Such is the case with musk ambrette, found in a number of cosmetic preparations. There have been several reports of CPS from musk ambrette (Raugi & Storrs, 1979; Raugi, Storrs & Larsen, 1979; Kroon, 1979; Giovinazzo *et al.*, 1980; Zugerman, 1981; Galosi & Plewig, 1982), and some of the patients developed PLR. Patients with PLR frequently show abnormal responses when their normal skin is tested to artificial radiation without the chemical being added. There is controversy as to whether this abnormal reactivity is restricted to UV-B (290–320 nm) or extends to UV-A

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(320–400 nm) (Ramsay, 1981). Patients with transient light reactivity are said to show normal responses to UV-B when their normal-appearing skin is tested without the chemical (Harber & Bickers, 1981).

This report describes five patients with CPS due to musk ambrette. Four of the five had PLR but one was a transient light reactor. Abnormal responses to artificial radiation were seen in all five patients when their normal skin was tested with artificial radiation.

METHODS

Skin tests with artificial radiation

These were always performed first. Normal skin on the back of the trunk that had not received deliberate exposure with any photosensitizing chemical was used. Tests were carried out with a solar simulator containing a 150 W Xenon lamp for patients 1–4 and with a Bausch and Lomb monochromator containing a 100 W Xenon lamp for patient 5. Radiant intensity of the solar simulator was measured with an International Light meter, IL 570, with appropriate detectors (SEE240 for UV-B and SEE 010 for UV-A). Multiple doses with the solar simulator were given between 5 mJ/cm^2 and 40 mJ/cm^2 for UV-B and a single dose of 6·5 J/cm^2 for UV-A. The radiant intensity of the source used for patient 5 was not measured because a meter was not available and doses were expressed in terms of exposure time. They were between 1 second and 60 seconds for UV-B, together with a single dose of 15 minutes for UV-A. All exposures were completed on one day and the results read after 20 minutes and after 24 hours.

The purposes of these tests were to determine the minimal erythema dose (MED) for UV-B, to detect an abnormal morphological response to UV-B, if present, and to detect the presence or absence of sensitivity to UV-A. Tests on normal individuals show that the doses of UV-A employed give no reactions. Thus a positive erythematous response to UV-A was considered abnormal and no attempt was made to determine the MED to UV-A. The MED to UV-B was the lowest dose in the series that produced erythema. In normal persons the UV-B doses employed only produced erythema. However, in some photo-sensitive subjects low doses of UV-B give an abnormal erythematous and papular response, and at times such a response is seen with the lowest dose of UV-B employed (Ramsay, 1979). Such a reaction to UV-B is reported here as an 'abnormal morphological response'. Thus sensitivity to UV-B may be manifested as a reduced MED or an abnormal morphological response at low doses or a combination of the two. With the sources used, the normal MED for the solar simulator was between 15 mJ/cm^2 and 40 mJ/cm^2 and for the monochromator between 6 seconds and 18 seconds.

Photopatch and patch tests

The following chemicals in petrolatum were used: bithionol (1%); tribrominated salicylanilide (1%); trichlorcarbanilide (2%); hexachlorophene (1%); dichlorophene (1%); *p*-amino benzoic acid (1%); musk ambrette (5%), and a control of plain petrolatum.

On day 1 the patches were applied in duplicate (sets A and B) on the back of the trunk. Set A was covered with light-impervious black paper. On day 2, set B was removed and the test sites were exposed to UV-A from two Phillips T20 BL 'UV-A' fluorescent lamps. On day 3, set A was removed and the results of both sets read. On day 5 the final reading for both sets was performed.

The dose of UV-A normally employed for photopatch tests was 2·5 J/cm^2 and this does not normally cause erythema. The dose was reduced to below that which did cause erythema in those patients known to be sensitive to UV-A. Without this precaution erythema from the UV-A fluorescent lamps would interfere with the interpretation of the photopatch tests.

TABLE 1. Summary of the results of photopatch and patch tests to musk ambrette and the response of normal skin to UVR. A single dose of 6·5 J/cm² of UV-A was delivered. EP = Erythematous and papular response. E = Erythematous response. Photopatch and patch test results given on a scale of + to + + + +

Case	Photopatch test	Patch test	UV-B Threshold dose	UV-B Morphology of threshold dose	UV-A
1	Positive + + +	Negative	< 5 mJ/cm²	EP	E
2	Positive + + +	Positive +	< 5 mJ/cm²	E (EP at 20 mJ/cm²)	E
3	Positive + +	Negative	< 5 mJ/cm²	EP	E
4	Positive + + +	Positive +	10 mJ/cm²	E (EP at 15 mJ/cm²)	Negative
5	Positive + + +	Negative	12 seconds	E (EP at 18 seconds)	Negative

CASE REPORTS AND RESULTS

Case 1. Male (aged 46 years)

He had vesicular hand dermatitis for several years treated with intermittent courses of intramuscular and topical corticosteroids. Early in 1979 he first developed a facial rash which persisted throughout the summer and was also present on sunny days in the winter. On one occasion early in 1980 there was a definite exacerbation after sun exposure, but except for this he was uncertain about the relationship between sun exposure and the rash. Apart from his steroid therapy there was no other medication. Examination revealed a few flat papules on the cheeks and sides of the neck. There was a mild vesicular dermatitis on the palms of both hands.

Skin tests with artificial radiation. The lowest dose of UV-B (5 mJ/cm²) showed an abnormal morphological response and the MED for UV-B was below 5 mJ/cm². An abnormal erythematous reaction developed to UV-A.

Photopatch and patch tests. There was a strongly positive photopatch test to musk ambrette. All other photopatch and all patch tests were negative.

Progress. Three sources of musk ambrette were detected. These were a shaving cream, a skin bracer and a medicated after-shave all from different manufacturers. PLR continued despite ceasing contact with musk ambrette.

Case 2. Male (aged 67 years)

This man had suffered from atopic dermatitis since childhood and there was a strong atopic family history. An eruption had recently developed on the face. Examination showed discrete, red, slightly scaly plaques on the forehead, cheeks and sides of the neck with one similar lesion in the lumbar region.

Skin tests with artificial radiation. The MED for UV-B was reduced (< 5 mJ/cm^2) and an abnormal morphological response occurred at 20 mJ/cm^2. UV-A produced an abnormal erythematous reaction.

Photopatch and patch tests. He developed a strongly positive photopatch test to musk ambrette. At the final reading there was a mildly positive patch test to musk ambrette. The remainder of the photopatch and patch tests were negative.

Progress. His source of contact to musk ambrette was a shaving cream, this was discontinued and the skin cleared. This patient had transient light reactivity.

Case 3. Male (aged 67 years)

This man immigrated to Canada from India in 1978. Several years previously in India he developed 'sensitivity to sunlight' that was thought to be due to a hair dye. After approximately 2 years his eruption subsided but it did not clear completely. In the 4 months prior to being seen his skin had become much worse with itchy red scaly plaques on the face and neck. Examination showed discrete red scaly plaques on the face and neck.

Skin tests with artificial radiation. An abnormal morphological response developed with the lowest dose of UV-B (5 mJ/cm^2); the MED for UV-B was < 5 mJ/cm^2. An abnormal erythematous response occurred with UV-A.

Photopatch and patch tests. There was a positive photopatch test to musk ambrette. All other photopatch and all patch tests were negative.

Progress. He had used several men's cosmetics but it was not possible to determine whether these contained musk ambrette. All contact with cosmetics ceased but despite this PLR continued.

Case 4. Male (aged 72 years)

In 1981 he developed a dermatitis on the left pinna that later appeared on the face and the dorsal aspects of the hands. The rash never occurred on skin covered with clothing. He was uncertain about exacerbation by sun exposure. The rash was worse in the summer but it persisted throughout the winter. Treatment with topical and systemic steroids had produced some benefit. He had used two brands of after-shave lotion but stopped soon after the onset of the rash because of the discomfort that occurred when the lotion was applied.

Examination revealed erythema and scaling on the left pinna, the sides of the neck, face, 'V' of the chest and dorsal aspects of the hands.

Skin tests with artificial radiation. The MED for UV-B was slightly reduced at 10 mJ/cm^2. An abnormal morphological response to UV-B was present at 15 mJ/cm^2. He did not react to UV-A.

Photopatch and patch tests. The photopatch test to musk ambrette was strongly positive. All other photopatch tests were negative. The patch test to musk ambrette was weakly positive. Patch tests performed previously elsewhere had shown positive reactions to musk ambrette, fennel and paraphenylenediamine.

Progress. The after-shave lotions used by him were not available, but we know that one of them had contained musk ambrette in the past. The PLR continued despite stopping the use of all topical agents except corticosteroids.

Case 5. Male (aged 57 years)

In the spring of 1978 he first developed an itchy scaly rash on his face. This waxed and waned in intensity but did not clear completely and was present throughout the summer and winter. On one occasion the rash became worse the day following exposure to fluorescent lamps at work and whilst playing a game of curling that same evening. Examination showed diffuse areas of erythema and scaling on the head and neck with a distribution that suggested a contact rather than a photocontact dermatitis. Investigations for photosensitivity were performed because of the definite history of exacerbation after exposure to fluorescent lamps.

Skin tests with artificial radiation. The MED for UV-B was normal (12 seconds), but with an 18-second exposure an abnormal morphological response occurred. He did not react to UV-A.

Photopatch and patch tests. There was a strongly positive photopatch test to musk ambrette. All other photopatch and all patch tests were negative.

Progress. The source of musk ambrette proved to be an after-shave lotion. Despite discontinuing the lotion PLR continued.

None of the patients demonstrated abnormal immediate reactions to artificial radiation. A summary of the results of the skin tests are shown in Table 1.

DISCUSSION

Contact photosensitivity resulting in PLR is less common now that many of the responsible chemicals have been removed from the market. However new photosensitizers are still being discovered and there is no doubt that musk ambrette can produce both transient and persistent photosensitivity. The clinical picture in four of the five patients reported here was suggestive but not typical of a light-induced eruption and in the fifth patient (case 5) a contact dermatitis was suspected. The true diagnosis was discovered in case 5 because of the patient's observation that his eruption was exacerbated by exposure to fluorescent lamps. The positive photopatch tests to musk ambrette confirmed the cause of the allergic photocontact dermatitis in all of the patients. In two (cases 2 and 4) patch tests to musk ambrette were also positive, although it is possible that these were examples of 'masked photopatch tests' (Epstein, 1963). The source of the musk ambrette was discovered with certainty in three patients (cases 1, 2 and 5), but it took persistence to obtain the information from the manufacturers of the toilet products. In one patient (case 4) the source of the chemical was suspected but not proven and in the final patient (case 3) we could not determine the source.

In patients with CPS the reactivity of the skin to artificial radiation can be investigated in two different ways so that two separate action spectra can be determined. The first action spectrum is that for the development of positive photopatch tests and this requires the presence of the chemical on the skin prior to the irradiation. This action spectrum has been measured for several different chemicals and has been shown to involve UV-A predominantly (Freeman & Knox, 1968; Cripps & Enta, 1970). A similar result was found with musk ambrette CPS in man and guinea-pigs (Giovinazzo *et al.*, 1981b). The second action spectrum in CPS in man is for the production of erythema on normal-appearing skin without the addition of the chemical. Published data are contradictory and the results vary depending upon whether patients with transient or persistent light reactivity are examined. It has been stated that sensitivity of the skin in transient light reactors is normal (Harber & Bickers, 1981; Giovinazzo *et al.*, 1981a). In PLR there is general agreement that UV-B produces an abnormal reaction (Wilkinson, 1962a; Jillson & Baughman, 1963; Masuda *et al.*, 1971; Freeman & Knox, 1968; Cripps & Enta, 1970; Hjorth & Wilkinson, 1968; Wilkinson, 1962b; Harber, Harris & Baer, 1966; Harber, Targovnik & Baer, 1967), although there is one report of a normal response to UV-B (O'Quinn, Kennedy & Isbell, 1967). Abnormal responses to UV-A are less well documented. UV-A sensitivity has been shown in some studies (Jillson & Baughman, 1963; Masuda *et al.*, 1971; Freeman & Knox, 1968; Ramsay, 1979), but not in others (Cripps & Enta, 1970; Harber *et al.*, 1967; Willis & Kligman, 1969; Epstein, Wuepper & Maibach, 1968).

Tests with artificial radiation on the normal skin of some patients with CPS from musk ambrette have been normal (Galosi & Plewig, 1982). Giovinazzo *et al.* (1980) reported two patients with PLR from musk ambrette; sensitivity to UV-B was present in both and to UV-A in one. The single patient with PLR presented by Zugerman (1981) was sensitive to UV-B but not to UV-A. Wojnarowska, Calnan and Hawk (1982) showed abnormal responses to UV-B and to UV-A in some patients.

The results of the present study show that there is no clear-cut pattern of UVR reactivity of normal skin in patients with CPS due to musk ambrette. These results also indicate that we must revise our ideas about the UVR response of normal skin in patients with transient light reactivity. Case 2 of the present series had this form of CPS and yet reacted abnormally to UV-B and UV-A. The other four patients had PLR from musk ambrette and all reacted abnormally to UV-B. Two of these patients (cases 1 and 3) also showed abnormal reactions to UV-A, but the other two (cases 4 and 5) did not. Studies on patients with transient and persistent light reactivity from other chemicals will be of interest to determine whether they also show these varied patterns of abnormal reactions to UVR.

The diagnosis of CPS may be difficult because the clinical features are not always typical, and the role of sunlight in the development of the eruption is not always realized by the patient. Whenever the diagnosis is suspected, skin tests with artificial radiation should be performed and these should be followed by photopatch and patch tests.

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Primary management of melanoma

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SUMMARY

A questionnaire was sent to all consultant dermatologists in The Netherlands, Belgium and Scotland regarding their management of suspected melanomas. The 343 evaluable responses showed that 29·7% of the dermatologists regularly prefer to 'wait and see' or carry out incisional biopsies in the early management of suspected melanoma, especially if the risk is thought low. The main arguments advanced by the proponents of incision were doubts about whether incisional biopsy provokes metastatic spread, and the belief that a pathologist can make reliable diagnosis on a partial specimen of the tumour. These arguments are questioned and excision biopsy, provided it is feasible without being disfiguring, is strongly recommended in the initial treatment of a suspected melanoma.

It is often stated that the cure rate of most cancers has not increased substantially over recent decades, but as far as malignant melanoma is concerned there has been a steady decline in the mortality rate since the first half of the century (Goldsmith, Shah & Kim, 1970). This favourable trend probably reflects the increased suspicion of both the public and doctors towards 'changing moles'. In Australia where the incidence is very high, widespread publicity and educational campaigns directed at recognizing early melanomas have resulted in survival statistics which are more favourable than elsewhere (Davis, 1976).

The treatment of advanced melanoma remains poor and, for the time being, efforts should focus on the development of educational and training programmes for the public, and doctors should encourage early and adequate treatment. Recently we found that family physicians, dermatologists and surgeons in The Netherlands regularly carry out punch or incisional biopsies where there is a suspicion of cutaneous malignant melanoma (Rampen & Rümke, 1984). Similarly, Macy-Roberts & Ackerman (1982) have shown that in the New York area referring specialists regularly perform partial biopsies on suspected melanoma prior to definitive treatment. As the dermatologist is often the first specialist to see a patient with a suspected malignant melanoma, we sent a questionnaire to dermatologists in three European countries enquiring about their preferred method(s) of primary management and the reasons for their choice.

METHODS

The questionnaire was sent in January 1983 to all practising dermatologists in The Netherlands, Belgium and Scotland. The following questions were asked:

(1) When did you finish your training as a dermatologist?

(2) At the institution where you were trained as a dermatologist, did they teach that no punch or incisional biopsy should be performed on a lesion suspected of being a melanoma?

(3) Do you presently practise at an academic institution or in general (office/private) practice?

(4) On average, how many melanomas do you see each year?

(5) What is (are) your preferred method(s) of primary management of suspected melanoma? Tumours on the face, hands and feet should not be considered. Indicate low (≤30%) and high (>30%) index of suspicion separately.

(6) If you regularly perform incisional procedures, what is the reason for choosing them?

(7) If you are opposed to incisional procedures, what defines this attitude?

RESULTS

Six hundred and sixty-eight dermatologists were sent questionnaires and 356 responded (The Netherlands 171/272; Belgium 161/369; Scotland 24/27). Thirteen answers were excluded from study because of (a) insufficient information on the preferred method of primary management (4) or (b) the dermatologists had retired from practice (9). Thus 343 replies were analysed.

Table 1 shows the preferred initial therapeutic approach according to geographical area and according to the index of suspicion of the lesion. Since many dermatologists indicated two or more methods of primary managements, the total number of methods used exceeds the total of dermatologists. Observation and incision (including incision biopsy, punch biopsy, shave biopsy and electrocautery) are often practised, especially with low suspicion lesions. In the high risk category, primary wide excision or immediate referral to a surgeon is the method of choice.

The numbers of dermatologists who regularly observe or carry out an incision biopsy on lesions of low clinical suspicion are indicated in Table 2. The figures for lesions of high clinical suspicion are not included because observation and incision were rare in this group (2·6%). The attitudes of dermatologists who trained more than 10 years ago and who trained within the past 10 years are analysed separately.

Young dermatologists in Scotland more often preferred observation and incision procedures

TABLE 1. Preferred method(s) of primary management in suspected melanomas

	Region (number of dermatologists) and index of suspicion							
	The Netherlands (163)		Belgium (156)		Scotland (24)		Total (343)	
	≤30%	>30%	≤30%	>30%	≤30%	>30%	≤30%	>30%
Initial management								
Observation	16	—	10	1	1	—	27	1
Incision	46	4	31	4	4	—	81	8
Narrow excision	64	8	84	11	13	2	161	21
Wide excision	22	46	52	84	2	4	76	134
Referral to surgeon	64	142	33	105	9	22	106	269

TABLE 2. Number of dermatologists observing or performing incisional biopsies on suspected melanomas according to year of registration. Percentages are given in parentheses

Region	Year of registration > 10 years ago		≤ 10 years ago		Total	
The Netherlands	30/78	(38·5)	27/85	(31·8)	57/163	(35·0)
Belgium	20/84	(23·8)	17/72	(23·6)	37/156	(23·7)
Scotland	1/14	(7·1)	4/10	(40·0)	5/24	(20·8)
Total	51/176	(29·0)	51/167	(30·5)	102/343	(29·7)

TABLE 3. Advice regarding melanoma management during the dermatological training as opposed to present attitude

Region	Number of evaluable cases	Advice during training: incisions allowed	Present attitude: incisions regularly performed
The Netherlands	151*	32 (21.2%)	42 (27·8%)
Belguim	156	21 (13·5%)	31 (19·9%)
Scotland	24	3 (12·5%)	4 (16·7%)

* Twelve unknown.

TABLE 4. Primary management by observation or incisional biopsy according to number of melanomas diagnosed during the last few years and type of dermatological practice. Percentages are given in parentheses

Type of dermatological practice	Number of melanonoma cases seen each year* 2 or less	3 or more	Total
Academic	7/27 (25·9)	8/51 (15·7)	15/78 (19·2)
Non-academic	49/153 (32·0)	34/109 (31·2)	83/262 (31·7)
Total	56/180 (31·1)	42/160 (26·3)	98/340 (28·8)

* Three unknown.

than their older colleagues, whereas the opposite was true for Dutch dermatologists. In Belgium there was no difference between these two categories.

The attitude at the institution of training is shown in Table 3. There was a clear correlation between what had been taught during dermatological training and the present attitude. The number of melanoma cases seen each year had no real impact upon melanoma management

TABLE 5. Arguments advanced for or against incisional biopsy

Dermatologists who regularly perform incisional biopsy ($n = 81$)		Dermatologists who never perform incisional biopsy ($n = 262$)	
The hazard of incisional biopsy regarding the risk of metasasis is exaggerated	37	Incision increases the risk of metastasis	216
The pathologist is able to make a clear diagnosis on partial specimen of the lesion	35	A partial biopsy may prevent proper histological diagnosis	120
Incision increases the risk of metastasis*	20	In most instances, whether benign or malignant, complete excision is desirable	12
A partial biopsy may prevent proper histological diagnosis*	11	Immediate radical excision is time-saving	9
Need to know the diagnosis before referring the patient to the surgeon	7	Other reason (less than five each)	16
Primary excision may be unduly mutilating or disfiguring	6	No reason mentioned	14
Other reason (less than five each)	16		
No reason mentioned	3		

* For discussion see text.

(Table 4). Dermatologists engaged in dermatological practice at a medical school or university, full time or part time, less frequently put their trust in observation or incision as compared with non-academic dermatologists (Table 4).

Table 5 shows the arguments put forward by the proponents of incision or excision to support their attitude. The ambivalence as regards the safety of incisional biopsy is clearly illustrated by the fact that several dermatologists believe that incision may have a detrimental influence upon the patient's survival (20) or upon proper histopathological reporting (11) but nevertheless regularly perform incisional procedures. It should be emphasized that dermatologists who rely on incisional biopsy only in cases of very low clinical suspicion are excluded from the incision category.

DISCUSSION

One of the dermatologist's prime roles in the management of suspected melanoma is to assess whether the lesion is likely to be malignant. Dermatologists see many pigmented and non-pigmented solitary lesions, some of which may be melanomas, where identification would be aided by biopsy. In the past decade incisional procedures have often been advocated as safe and reliable, a view supported by the work of Epstein, Bragg & Linden (1969), who noted a similar survival rate in patients with immediate radical surgery and in those with a pretreatment biopsy. Their study was a welcome contribution at a time when old dogmas were responsible for too frequent and drastic procedures like amputation and hemipelvectomy for primary melanoma, and a change towards a more conservative therapy was undoubtedly warranted. However, most adherents of the 'biopsy' tenet have erroneously translated the findings of Epstein *et al.* as an excuse to perform incisional or punch biopsies. Epstein *et al.* did not mention the words 'incision' and 'punch' biopsy at all. They only refer to 'biopsy' as a limited approach as opposed to immediate radical excision. Their biopsy category comprised incisional as well as excisional procedures.

Incisional biopsy and punch biopsy are fraught with considerable hazard and the statement that there appears to be no evidence of harm from punch or incisional biopsy no longer holds true. Rampen, van Houten and Hop (1980) have shown that incisional procedures are associated with a significant decrease in survival rate. They also cited a presentation by Van Slooten and Van der Esch at the IVth International Symposium on Malignant Melanoma in Lyon (1977), indicating that incisional biopsy foreshadows an unfavourable outcome. Confirmation of these observations would lead to refutation of the myth that incisional biopsy is an acceptable method for the diagnosis of melanoma.

Another drawback of incisional procedures has not received much attention in the literature: the risk of local recurrence. Tumour cells may be forced into the surrounding and deeper tissues. This tumour cell spill may enlarge the original tumour-bearing area considerably. What initially was a Clark II or III melanoma may theoretically be converted into a Clark IV or Clark V melanoma. Locations where the underlying 'buffer-zone' of subcutaneous fat or muscular fascia is lacking are expecially at risk. This may partly explain the poor survival of melanoma patients with primaries on the scalp, midline of trunk, hands and feet, in the recent series of Rogers *et al.* (1983).

Furthermore, complete excision aids reliable histopathological reporting, which is only possible when the entire lesion is available for assessment (Macy-Roberts & Ackerman, 1982). This is especially important with regard to the subtle differences between precursor lesions (e.g. dysplastic naevi) or Spitz's juvenile melanomas and early malignant melanomas. Accurate microstaging by Breslow's method (1970), presently the most powerful prognostic determinant, is only possible when the full specimen is available to the pathologist.

Our questionnaire shows that many dermatologists seem prepared to risk incisional procedures for suspected melanomas on areas other than the face and digits. To us, however, it seems prudent to stick to excisional biopsy, whenever feasible, with a tumour-free resection margin of 0·5–1 cm; these excisions may be carried out under 'field block' anaesthesia. A narrower excision may be advisable in selected cases, depending on suspicion rate, location of the tumour and practicability of primary closure of the wound. If one is almost certain a large lesion is malignant, immediate wide excision (margin 3–5 cm) under general anaesthesia may be expedient, especially where primary closure of the diagnostic excision cannot be achieved. In such cases, a frozen section may be reported, followed by wide radical excision under the same anaesthesia if the limited excision confirms the diagnosis of melanoma.

We should emphasize that we do not wish to be dogmatic about incisional biopsy. The three risk factors associated with incision into the tumour (inaccurate histological assessment, metastatic potential, local recurrence) are not absolute. The pathologist may be able to provide a reliable diagnosis in many instances if only part of the growth can be assessed but a fragment of a small or early lesion will often cause a great strain on the most experienced pathologist. As to metastasis and local recurrence it is likely that most tumour cells which are dislodged by the incision will perish before they can settle at near or remote sites. If, however, only a small percentage of these cells is able to survive and give rise to metastatic tumour burdens, then incision is to be strongly discouraged. We believe that patients with (suspected) melanomas should be offered the best chance of survival. In this philosophy there is no place for iatrogenic risk factors like incisional or punch biopsy, as long as excision is feasible without being mutilating or disfiguring.

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Studies of the clinically uninvolved skin in patients with dermatitis

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SUMMARY

We have studied the clinically normal skin of eighty-four patients with quiescent dermatitis of different types, and fifty normal subjects. Increased susceptibility to chemical irritation was demonstrated in patients with atopic and exogenous dermatitis. There was a reduction in mean corneocyte area and a thinner horny layer in all patients, and an increased epidermal thickness in atopic dermatitis patients only. In forced desquamation studies, patients released more corneocyte clumps from the skin surface, but there were no significant differences between patients and controls in sensitivity to UVR or electrical conductivity.

Substances responsible for contact dermatitis must penetrate the stratum corneum to reach the vulnerable tissues beneath, and the question arises as to whether there is an inherent abnormality of the horny layer in individuals who have a history of dermatitis.

The aim of this study was to compare stratum corneum structure and function in the uninvolved skin of patients who have previously had contact dermatitis with that of uninvolved skin of patients with quiescent constitutional dermatitic disorders and with the skin of healthy subjects.

METHODS

All the patients and controls were Caucasian subjects who had given their informed consent and had attended the dermatology clinic of the University Hospital of Wales, Cardiff. A total of eighty-four patients with a recent history of mild or moderately severe dermatitis of different types were investigated (Table 1). None had a history of other skin disorders or systemic disease. Diagnosis of dermatitis in the different categories was made clinically according to published criteria as follows: atopic dermatitis (Sulzberger, 1975), contact dermatitis (Vickers, 1975), dyshidrotic eczema (Rook & Wilkinson, 1979), lichen simplex chronicus, asteatotic dermatitis, hand dermatitis and seborrhoeic dermatitis (Ryan, 1975) and nummular dermatitis (Soter & Fitzpatrick, 1979).

At examination the dermatitis process of all patients was in an inactive stage. None of the areas of skin investigated were being treated with any topical medicament. Note was made of the depth of skin pigmentation and the usual reaction to sun exposure.

The controls consisted of two groups of subjects: (1) patients with minor localized skin disorders (such as seborrhoeic warts or basal cell carcinoma) and without any systemic disease, and (2) normal healthy individuals. All the subjects investigated were interviewed and then examined.

Tests employed

Minimal erythema dose of UVR (MED). The MED was determined by irradiating the skin with a UV lamp (Westinghouse FS20 fluorescent tubes, peak emission 313 nm). The lamp was placed perpendicularly over the skin at a distance of 45 cm from the right side of lower back. The least time required to produce erythema after 24 hours, under the standard conditions employed, was recorded.

Ammonium hydroxide minimal blistering time (MBT). The procedure was based on that described by Frosch and Kligman (1977). Application of a 50% aqueous solution of ammonium hydroxide (7·7 M solution) to the skin leads to blister formation. The solution was applied to the skin of the left forearm ventral aspect, by means of a small perspex chamber, and the time to appearance of small blisters was recorded. It has been observed that the MBT is related to the intensity of dermatitis provoked by exposure to sodium lauryl sulphate and has been used as an index of barrier function and skin sensitivity to irritants (Frosch & Kligman, 1977).

Forced desquamation. Mechanical stimulation of the skin surface in the presence of a non-ionic detergent (phosphate-buffered 0·1% Triton X-100) leads to the release of a large number of corneocytes. Using a specially designed apparatus (the Desquamator, Cutech, Ltd.) which delivers a variable and measured rotational stimulus to the skin surface for a measured time, it is possible to obtain a measure of the numbers of corneocytes shed on non-stimulated skin (Roberts & Marks, 1980). Measurements were performed on the volar aspect of the left forearm and the number of cells released were counted using a Neubauer haemocytometer.

Electrical conductivity. The electrical conductivity of the stratum corneum (SC) of the left ventral forearm was measured using a device described by Tagami *et al.* (1980) (Conductance Capacitance Meter, Model IB355, I.B.S. Inc., Hammanatsu, Japan). The electrical conductivity determined with this device is said to be closely related to the water content of the SC and to its functional integrity.

Corneocyte surface area (mean corneocyte area—MCA). The MCA was determined using a microscope projection technique. Samples of detergent containing corneocytes were obtained using the desquamator described previously and these were smeared on to glass microscope slides. A minimum of thirty corneocytes were assessed to obtain the MCA.

Stratum corneum thickness and epidermal measurements. Small 'punch' biopsies (3 mm diameter) were removed under local anaesthesia from the outer, upper aspect of the left arm. The biopsies were cryostat sectioned and the thickness of the SC was measured. Absolute thickness was determined using a microscope projection technique (after staining with haematoxylin and eosin) and in terms of the numbers of cell layers (after staining with methylene blue and expansion in Sörensen Walbum buffer solution at pH 11·5).

Measurements of mean epidermal thickness (MET) were obtained from the haematoxylin and eosin stained sections by means of a projection technique (Marks, 1981). The ratio of the length of the basal layer to the length of the granular (B/G ratio) was also estimated using a map curvimeter on the drawn projected image.

All the investigations were performed in a quiet, draught-free room. The ambient air temperature ranged from 19°C to 22°C and the relative humidity ranged from 45% to 55% during the studies.

Statistical methods

The patients studied were divided into three group: (I) atopic dermatits, (II) other forms of constitutional dermatitis and (III) contact dermatitis (Table 1). Comparisons were made firstly between each group and the controls, secondly between all the patients with the various forms of dermatitis and the controls, and thirdly between patients with contact dermatitis and those with constitutional dermatitis.

The analyses were performed in the Department of Medical Statistics at the Welsh National School of Medicine, Cardiff, using the computer package SPSS (Statistical Package for the Social Sciences—Nie *et al.*, 1975). Retrospective statistical matching was performed (adjusted analysis) and the effect of sex, skin type and age on the variables examined was taken into consideration. Matching was performed by unbalanced analysis of variance. The adjusted means were obtained from the multiple classification analysis and the residual standard deviations from the analysis of variance.

Most of the variables except the MED, MBT and MCA are slightly positively skewed; they are more normally distributed if log transformed.

The analyses were performed on the untransformed data of all the variables, and then repeated on the log-transformed data of the six slightly skewed variables. In order to show the actual values of the latter variables, the untransformed data are presented in the Tables, and

TABLE 1. Diagnoses of patients studied and numbers and ages and controls

Group	No.	Group total	Age range	Mean age ± s.d.
I Atopic dermatitis		22	17–46	24·9±7·3
II Other forms of constitutional dermatitis		32	18–72	43·1±15·5
Lichen simplex chronicus	4			
Dyshidrotic eczema	9			
Nummular eczema	2			
Asteatotic eczema	2			
Seborrhoeic eczema	8			
Hand eczema (non-contact)	7			
III Contact dermatitis		30	17–72	36·2±16·2
Irritant	13			
Allergic	11			
Mixed irritant and allergic	6			
All patients		84	17–72	35·9±16·0
Controls		50	19–73	43 3±17·7

whenever the log-transformed data of these variables show a different pattern of statistical significance, it is indicated accordingly.

RESULTS

Sensitivity to UVR

The mean MEDs for controls, patients with atopic dermatitis and those with contact dermatitis were 2·73 ± 0·72, 2·28 ± 0·72 and 2·45 ± 0·72 min respectively. The differences were not significant. There were more subjects with type I and type II skin in the patient group than in the control group but these differences were not significant.

Minimal blistering time to applied ammonium hydroxide

All the groups of patients showed a lower MBT when compared with control subjects (Table 2). Patients with atopic dermatitis and contact dermatitis were significantly different compared with controls. They had the lowest value but this was not significantly different from the values for the other groups of dermatitis patients.

Electrical conductance

There were no significant differences between the mean conductances of any of the patient groups and the control subjects (controls 11·77 ± 4·74, atopic dermatitis 9·0 ± 4·74, contact dermatitis 10·35 ± 4·74 $\mu\Omega$).

Corneocyte and stratum corneum measurements

In Table 2 it can be seen that the mean corneocyte area values are significantly decreased in all the groups of patients with dermatitis compared with controls. The lowest value is for patients with atopic dermatitis. Table 2 gives the value for SC thickness. Values of SC thickness of the patient groups in absolute terms are lower than that for controls, but statistical significance ($P < 0{\cdot}05$) is reached only when the combined values of log-transformed data from all patients

TABLE 2. Mean blistering time and stratum corneum (SC) measurements in patients and controls

	Minimal blistering time			Mean corneocyte area values			SC thickness values‡		
Subject groups	*n*	Mean ± s.d. (min)	Sig.†	*n*	mean ± s.d. (μm^2)	Sig.†	*n*	Mean ± s.d. (μm)	Cell layers
Atopic dermatitis	22	12·0 ± 4·04*	*P* < 0 05	22	891 3 ± 102·67*	*P* < 0·001	33	12·3 ± 3·94*	13·3 ± 2·64*
Constitutional dermatitis	32	12·9 ± 4·04*	NS	32	923 0 ± 102 67*	*P* < 0·001	31	14·3 ± 3 94*	13·6 ± 2·64*
Contact dermatitis	30	12·4 ± 4·04*	*P* < 0·05	30	914·3 ± 102·67*	*P* < 0·001	28	13·6 ± 3·94*	13·2 ± 2·64
Controls	45	14·8 ± 4·04*		50	1022·4 ± 102·67*		49	15 1 ± 3·94*	13·9 ± 2·64*

* Pooled (residual standard deviation).

† Significance compared with controls.

‡ There were no significant differences in either of these columns.

TABLE 3. Epidermal thickness and B/G ratios of patients and controls

Subject groups	Number	Mean ± s.d.*	Significance compared with controls
(a) *Comparison of epidermal thickness values (μm) between patients and controls*			
Atopic dermatitis	17	49·54 ± 12·04	$P<0{\cdot}01$
Constitutional dermatitis	31	59·26 ± 12·04	NS
Contact dermatitis	27	62·65 ± 12·04	NS
Controls	49	57·42 ± 12·04	
(b) *Comparison of B/G ratios between patients and controls*			
Atopic dermatitis	17	1·190 ± 0·118	$P<0{\cdot}001$
Constitutional dermatitis	31	1·160 ± 0·118	$P<0{\cdot}01$
Contact dermatitis	27	1·130 ± 0·118	$P<0{\cdot}01$
Controls	49	1·060 ± 0·118	

* Pooled (residual) standard deviation.

(13·64 ± 3·90) is compared with that of controls (15·02 ± 3·90). There are no differences in SC thickness as far as values for SC cell number are concerned.

Rate of corneocyte loss during forced desquamation

No significant differences were observed in the patient groups compared with controls. However, there was a marked difference ($P<0{\cdot}001$) in the numbers of subjects showing corneocyte clumping in the detergent used to scrub the skin surface. Seventy-seven of the eighty-four patients showed clumping, compared with thirty-three of the fifty controls ($\chi^2 = 15{\cdot}46$, $P<0{\cdot}001$).

Epidermal measurements

Epidermal thickness was increased in atopic dermatitis patients (Table 3a) but in no other group compared with controls. The B/G ratio was significantly increased in all patient groups compared with the control subjects and the increase was greatest in patients with atopic dermatitis (Table 3b).

DISCUSSION

The tests performed in this study were intended as a screen for easily detectable abnormalities in the uninvolved skin of patients with eczema. Only some of the tests performed demonstrated significant differences in patients' skin compared with that of controls and in most respects the uninvolved skin of patients with quiescent dermatitis of the types investigated must be considered normal from both the structural and functional viewpoints. Findings of particular interest included an increased susceptibility to irritation with ammonium hydroxide solution in patients with dermatitis (lower threshold exposure time than controls) and a significantly reduced mean corneocyte area in uninvolved skin of patients with dermatitis. Abnormally small corneocytes may be linked with defective barrier function as many scaling diseases have been shown to be characterized by abnormally small cells and an altered barrier. Similarly, in normal healthy individuals, areas of skin with small corneocytes (e.g. palms, soles, facial skin) demonstrate increased transepidermal water loss and increased intracorneal cohesion. This variation in barrier function in the presence of small corneocytes may be related to the increased intercellular volume per unit volume of stratum corneum (Marks, Nicholls & King, 1981a). This is also in agreement with Elias (1981) who suggested that the intercellular pathway was important in percutaneous transport. Furthermore, the previously observed significantly positive relationship beteeen mean corneocyte area and MBT also supports the above hypothesis (Al-Jaberi, 1983).

The quality and rate of maturation of the horny layer are other factors to which a defective barrier may be attributed. The numbers of clumped corneocytes released after scrubbing the skin surface of the uninvolved skin of dermatitic patients were found to be significantly higher compared with controls. 'Clumping' as seen on the microscope slide probably has the same pathophysiological significance as scaling clinically and indicates a failure of the normal loss of binding forces between horny cells at the skin surface. The phenomenon of 'clumping' has been observed previously in patients with a variety of scaling disorders (Marks *et al.*, 1979; King *et al.*, 1979; Finlay *et al.*, 1980), characterized by increased epidermal proliferative activity, decreased transit time and consequent relative immaturity of the horny cells or dysmaturation as in the ichthyotic diseases.

The findings presented here suggest that the clinically normal skin of patients with all forms of dermatitis may be functionally abnormal. This may partially explain the 'angry back syndrome' and help to explain the appearance of dermatitic rashes at distant sites after relatively minor provocation in patients with dermatitis starting elsewhere. The altered SC in the group of patients with contact dermatitis may have special aetiological implications and could form the basis of predictive testing for the development of contact dermatitis in 'high risk occupations'. Clearly, further work is required to characterize the fault in these 'dermatitis prone' subjects.

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Pharmacology and Treatment

Superficial X-ray therapy in the treatment of constitutional eczema of the hands

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SUMMARY

Twenty-four patients with chronic symmetrical constitutional eczema of the hands were treated with a combination of topical therapy and conventional superficial X-ray therapy. In a double-blind fashion one hand was irradiated with 100 rad (1 Gy)* at 50 kV on three occasions at intervals of 21 days and the other hand was given a placebo treatment. Each hand received the same topical medication, which was adjusted as necessary at each visit. The patients were seen 6, 9 and 18 weeks after X-ray therapy commenced. A significantly better therapeutic result, as assessed independently by both the patient and the observer, was recorded on the hand which had received active X-ray treatment. The advantage bestowed by active X-ray therapy was greatest 6 to 9 weeks after the start of treatment, but was still present after 18 weeks.

The therapeutic advantage gained by combining conventional superficial X-ray therapy with topical therapy has not previously been assessed in an adequately controlled trial. Since the advent of potent topical steroids many dermatologists have abandoned the use of X-ray therapy in the management of benign dermatoses. However, some benign dermatoses are relatively resistant to treatment with topical preparations. The present double-blind placebo-controlled trial was designed to assess the efficacy of conventional superficial X-ray therapy in the treatment of constitutional eczema of the hands. The exposure dose, kV and fractionation of the X-ray therapy is the standard regime which has been used in the Department of Dermatology at the General Infirmary at Leeds for several years.

METHOD

Twenty-four patients with chronic symmetrical constitutional eczema of the hands took part in

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* Dermatologists in the past have used the roentgen (R) unit which is a unit of exposure. The absorbed dose is of more biological relevance than the exposure dose and articles now use the term rad as the unit of absorption. It is difficult to measure the absorbed dose in tissue, but the absorbed dose is proportional to the exposure at the same point. In the doses used in superficial radiotherapy the difference between a roentgen and a rad is not significant. Using SI units one gray (Gy) equals 100 rad.

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the trial. Cases of contact allergic and irritant dermatitis were excluded by taking a careful history and, where necessary, by patch testing. Patients continued treatment with tar paste or steroid ointments to both hands throughout the trial. Each hand received the same topical therapy but only one hand was treated with active conventional superficial X-ray therapy. The other hand received a placebo therapy. The radiographer (D.P.M.) treating the patient gave the active radiation or placebo treatment according to a predetermined code. The observer (G.M.F.) did not know which hand was receiving X-ray therapy until the code was broken at the end of the trial. During the X-ray treatment the patient wore a lead apron extending from the knees to the neck. The X-ray beam was directed on to the skin using a lead applicator which gave a focal skin distance of 30 cm. The open end of the applicator rested on the skin, limiting X-ray exposure to the eczematous area. One hand was irradiated with 100 rad (1 Gy) at 50 kV through a 1 mm aluminium filter using a Dermopan 11 X-ray machine to give 50% absorption of X-ray at a depth of 13 mm in the skin (half-value layer 0·85 mm of aluminium). The other hand received a placebo X-ray treatment. The X-ray machine appeared to function normally during the placebo treatment, but it had been specially adjusted so that the electricity supply to the X-ray tube was interrupted when the dial on the filter head was moved into position two. This manoeuvre could be performed by the radiographer without the patient's knowledge. The X-ray therapy was given on three occasions at intervals of 21 days.

The eczema was assessed, using three different methods, 6, 9 and 18 weeks after starting X-ray therapy. The patient was asked at each visit which hand had improved more since treatment commenced. The observer made a graded assessment of each hand according to the following system:

Grade 0	Normal looking skin
Grade 1	Mild scaling and erythema
Grade 2	Moderate scaling, erythema and shallow fissures
Grade 3	Severe scaling, erythema and deep bleeding fissures
Grade 4	Active pompholyx

The patient also graded the severity of the eczema on each hand on a scale between 0 and 10. When the trial was complete the irradiated side was identified. The results of the patients' preferences were analysed using McNemar's test. The results from both grading systems were analysed using Student's *t*-test.

RESULTS

One subject, who showed a superior result on the irradiated side, failed to keep follow-up appointments at 9 and 18 weeks. This subject's results were not included in the analysis of the findings. Thirteen subjects received irradiation on the dominant and ten subjects on the non-dominant hand and this did not appear to influence the results of the X-ray treatment. No systemic or local adverse reactions were noted.

The patients' opinion of improvement is shown in Table 1. A greater improvement on the hand receiving active X-ray treatment was recorded significantly more often at 6 and 9 weeks ($P < 0{\cdot}01$; $P < 0{\cdot}05$). The assessment made 18 weeks after X-ray therapy had been commenced showed that nine patients still preferred the therapeutic result on the irradiated side ($P < 0{\cdot}05$). The results of the grading system used independently by the observer and the patient are shown in Figures 1 and 2 respectively. The observer recorded that the mean score of the eczema on the hand receiving active X-ray treatment was significantly lower than the mean score of the eczema on the hand receiving placebo X-ray treatment 6, 9 and 18 weeks after X-ray therapy had been commenced ($P < 0{\cdot}001$; $P < 0{\cdot}001$; $P < 0{\cdot}01$). The patient recorded similar findings. The mean score of the eczema on the irradiated hand was significantly lower at each visit ($P < 0{\cdot}01$; $P < 0{\cdot}01$; $P < 0{\cdot}05$).

TABLE 1. Patient assessment of X-ray treatment

	Week 6		Week 9		Week 18	
Greater improvement on irradiated hand	19	$P < 0{\cdot}01$	16	$P < 0{\cdot}05$	9	N.S.
Greater improvement on placebo-irradiated hand	3		2		2	
Equal changes on both hands	1		5		12	

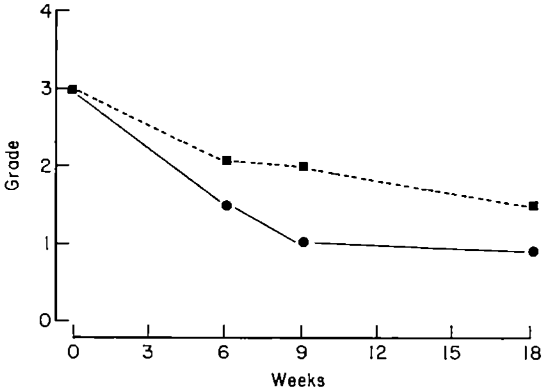

FIGURE 1. Observer's mean grade of the eczema on the X-ray treated hands (●——●) and on the placebo-treated hands (■ - - - ■), ± standard error of the mean.

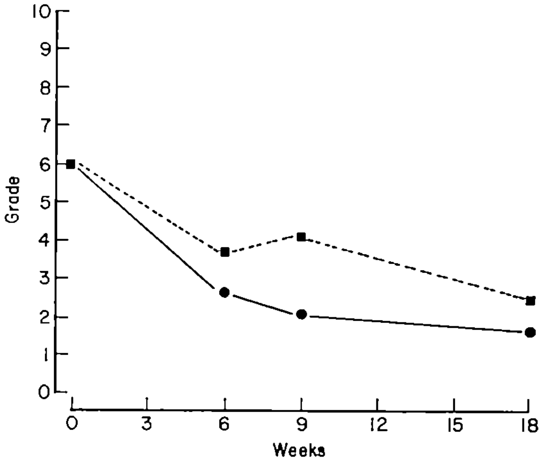

FIGURE 2. Patients' mean grade of the eczema on the X-ray treated hands (●——●) and the placebo-treated hands (■ - - - ■), ± standard error of the mean.

DISCUSSION

The combination of topical medication and conventional X-ray therapy proved superior to topical medication alone in the treatment of constitutional eczema of the hands. Three hundred rad (3 Gy) at 50 kV, fractionated into three equal doses over a 6-week period produced a greater reduction in the mean grade of the eczema on the irradiated hand, than on the hand receiving placebo X-ray treatment. The therapeutic benefits of X-ray therapy were most marked 6 to 9 weeks after the start of treatment but were still present 18 weeks after X-ray therapy had been initiated. The major effect of conventional superficial X-ray therapy is an increased rate of healing and this is particularly important to a person who is absent from work and whose income is diminished because of hand eczema.

A previous trial has shown that conventional superficial X-ray therapy used alone, without topical medication, has a therapeutic effect upon eczema (Crissey & Shelley, 1952). However, the X-ray exposure was larger than that which would be used in clinical practice today. Frain-Bell and Bettley (1959) compared the effects of the combination of topical medication and 1200 rad (12 Gy) of Grenz ray with those of topical medication alone in cases of symmetrical eczema. The Grenz ray therapy was fractionated into three doses over 3 weeks and was found to be effective only while it was being administered. When the patients were reviewed 4 to 8 weeks later there was no difference between the eczema on the two sides. Thus conventional superficial X-ray therapy appears to be more effective than Grenz ray therapy, and we are currently investigating this point.

The dermatological use of X-ray therapy for benign disorders has declined in recent years. Sweet (1968) suggested that the use of X-ray therapy should be abandoned except for the treatment of malignant conditions because of the potential risks. However, Rowell (1973) showed that X-ray therapy is not associated with any long-term complications providing that less than 1200 rad (12 Gy) of conventional superficial X-ray therapy are given to any site during the patient's lifetime. These findings are in agreement with those of an earlier study (Sulzberger, Baer & Borota, 1952) which also suggested X-ray therapy should be avoided in people who are exposed to excessive amounts of sunlight. It is now recommended that the total lifetime exposure to conventional superficial X-ray therapy of any unit of skin should not exceed 1000 rad (10 Gy) (Rowell, 1978). In practice this means that a patient could have three courses of 300 rad (3 Gy) in a lifetime. Patients should be warned about the danger of exceeding this so that they do not go to different centres for further radiation. X-ray therapy to the skin in the region of the thyroid gland may increase the risk of developing carcinoma of the thyroid (Winship, 1956). Today, however, the thyroid gland is shielded by lead on all occasions. A recent paper has estimated that X-ray therapy to the hand, given in the manner described in this paper, would be associated with approximately a 1 in 2 million chance of inducing a carcinoma of the thyroid gland and a mortality risk of less than 1 in 50 million (Goldschmidt, Gorson & Lassen, 1983). Taking only a 1 in a million risk is the equivalent of reducing one's life expectancy by 8 minutes, or smoking 1·4 cigarettes, travelling 6 miles by canoe, 10 miles by bicycle, 300 miles by car or 1000 miles by jet aircraft (Wilson, 1979). It can be concluded that there is no appreciable long-term risk associated with the correct use of conventional superficial X-ray therapy as described in this paper. Severe X-ray burns have occurred in the past when patients have inadvertently been given an overdose of X-ray therapy (Sweet, 1962) but modern X-ray machines have built-in safety devices that reduce the risk of such an operator error occurring.

Our study indicates that patients with constitutional eczema of the hands, which is not improving with the use of topical medication alone, benefit from the administration of a course of conventional superficial X-ray therapy. This therapy is not associated with any appreciable long-term risk.

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A double-blind study of superficial radiotherapy in chronic palmar eczema

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SUMMARY

The effect of superficial radiotherapy as an adjunct to topical therapy in recalcitrant chronic symmetrical palmar eczema was assessed in fifteen patients by randomly allocating active treatment to one palm while the other, which received simulated therapy, served as a control. There was a significantly better response to active treatment at 1 month but this difference was no longer apparent at 3 and 6 months.

Superficial radiotherapy has long been an important part of dermatological therapy but its popularity has declined in the United Kingdom since the introduction of potent topical corticosteroids, particularly because of fears of long-term sequelae (Rowell, 1978). It is however still widely used in Europe (Lukacs, Braun-Falco & Goldschmidt, 1978) and North America (Goldschmidt, 1975a) as an adjunct to topical treatment in benign dermatoses. Forty-one per cent of 2444 American and Canadian dermatologists questioned by Goldschmidt used Grenz or superficial X-rays regularly; approximately one-third used superficial radiotherapy in chronic eczema and lichen simplex.

Despite its widespread use, superficial radiotherapy has been evaluated in only one controlled study (Fairris, Mack & Rowell, 1984), although it has been claimed to be effective in chronic eczema (Brown, 1958; Lukacs *et al.*, 1978). Chronic palmar eczema is often particularly difficult to control with topical tar or corticosteroids and it is the most common benign dermatosis for which superficial radiotherapy is used in Manchester. In order to assess its efficacy we carried out a controlled study to compare it with a sham procedure in patients with chronic symmetrical palmar eczema.

METHODS

Twenty patients with chronic symmetrical palmar eczema which was unresponsive to topical steroids or tar and which had been stable for at least 3 months entered the trial. Subjects who were pregnant, under 25 years of age, who were receiving oral steroids or cytotoxic agents or

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who had a history of skin neoplasia or previous radiotherapy were excluded from the study. The trial was approved by the District Ethical Committee and signed informed consent was obtained from each patient. The type of eczema and the current topical therapy were recorded. At the first and each subsequent visit the extent of the lesions was sketched in the case notes and photographs were taken. The eczema was categorized as 'clear', 'nearly clear', 'partly clear', 'no change' and 'relapse'. Topical medication was continued unchanged during the trial.

Superficial radiotherapy was administered with a Philips RT 100 machine using a 0·55 mm aluminium filter and a focal distance of 30 cm. Three fractionated doses of 100 rad (cGy) at 45 kV were given at 1-week intervals. The radiographer randomly selected one palm for active treatment while simulated radiotherapy was given to the other palm which served as a control. Neither patient nor doctor was aware which hand had received treatment, but a record was kept by the radiographer in a sealed envelope. At the end of the study the radiotherapy records were examined and the results of active and sham treatments were compared using Fisher's exact test. The patients were reviewed 1 month, 3 months and 6 months after completion of therapy. The responses were graded as 'clear' or 'nearly clear' indicating a good response, while 'partly clear', 'no change' or 'relapse' indicated a poor response.

RESULTS

Twenty patients entered the trial; three defaulted from follow-up and two were excluded because their topical therapy was changed during the study. Of the fifteen who completed the trial, eight had hyperkeratotic eczema and seven had chronic pompholyx eczema.

One month after completion of radiotherapy seven patients (46%) showed a good response on the treated side but none of the untreated palms had improved (Table 1a). This difference was statistically significant ($P < 0{\cdot}01$). Of the seven patients who responded well, five had hyperkeratotic eczema (Table 1c).

By 3 months ten patients (66%), of whom seven had pompholyx eczema (Table 1b), had

TABLE 1. Response of patients with chronic palmar eczema, pompholyx eczema or hyperkeratotic eczema

	Treated palm		Untreated palm	
	Good response	Poor response	Good response	Poor response
(a) *Chronic palmar eczema* (*n* = *15*)				
1 month	7	8	0	15
3 months	10	5	6	9
6 months	11	4	8	8
(b) *Pompholyx eczema* (*n* = *7*)				
1 month	2	5	0	7
3 months	7	0	3	4
6 months	7	0	6	1
(c) *Hyperkeratotic eczema* (*n* = *8*)				
1 month	5	3	0	8
3 months	3	5 (3 relapses)	3	5
6 months	4	4	2	6

shown a good response on the treated side, but in five of these the untreated side had also improved. In one patient the untreated palm cleared but there was no improvement in the treated palm. Three of the five patients whose hyperkeratotic eczema had responded at 1 month had relapsed by this time (Table 1c).

At 6 months eleven (74%) of the treated palms and eight (54%) of the untreated palms were clear or nearly clear. The difference between treated and untreated palms did not reach significance.

DISCUSSION

Superficial radiotherapy is safe provided that the dose is fractionated and that no more than 1200 rad are given to any one area of skin in a lifetime (Rowell, 1972). Because of their thick stratum corneum, the palms and soles are the least radiosensitive parts of the body (Kalz, 1941). Moreover, a follow-up study of 122 patients who had received radiotherapy for hyperhidrosis between 2 and 18 years previously showed no chronic sequelae on the palms after cumulative doses of up to 1600 rad (Borak, Eller & Eller, 1949). Similar findings were reported by Miescher and Böhm (1948).

If superficial radiotherapy is safe, is it an effective treatment for benign dermatoses? Chronic eczema and lichen simplex are the most common benign conditions for which radiotherapy is used (Goldschmidt, 1975b). Total doses varying from 240 rad (Brown, 1958) to 400 rad (Lukacs *et al.*, 1978) have apparently met with success. Fairris *et al.* (1984) performed a double-blind trial of the effect of 100 rad at 50 kV on three occasions at intervals of 21 days, and showed that superficial radiotherapy produced a significant improvement which was greatest 6 to 9 weeks after the start of treatment.

We used weekly doses of 100 rad at 45 kV to a total of 300 rad. This regime was beneficial at 1 month, but by 3 months the benefit was less certain and could not be demonstrated statistically in our small sample. By 6 months it appeared that pompholyx eczema had resolved whether radiotherapy had been given or not. Any difference which had been apparent at 1 month in the patients with hyperkeratotic eczema had disappeared by 6 months. Radiotherapy thus seemed to have a definite but limited and temporary effect.

Our regime used X-rays of relatively low penetration and further studies would be needed to show whether more penetrating X-rays such as used by Brown (1958) and Lukacs *et al.* (1978) would produce significantly better results. Our study, however, suggests that the benefits of superficial radiotherapy in chronic palmar eczema do not materially alter the course of the disease.

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Short-contact modification of the Ingram regime

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SUMMARY

'Minutes' (30 minutes) and 'short-contact' (2 hours) therapy with dithranol in Lassar's paste were compared (using a paired comparison method) with the Ingram regime in twenty-one and twelve patients respectively. Both 'minutes' and 'short-contact' regimes were effective in clearing psoriasis; the latter was the more effective of the two and proved just as good as the standard Ingram regime of 24-hour application. 'Short-contact' therapy with dithranol in Lassar's paste should therefore reduce out-patient attendance and time off work, save valuable nursing time and allow a more social life.

The efficacy and safety of the Ingram regime (Ingram, 1953) which relies on the 24-hour application of dithranol in the treatment of psoriasis is well established. Application of dithranol for a shorter duration is effective (Runne & Kunze, 1982; Marsden *et al.*, 1983), but the base is important. Dithranol in yellow soft paraffin (YSP) (Statham, Ryatt & Rowell, 1984) or emulsifying ointment (EO) based preparations (Marsden *et al.*, 1983), although effective in clearing psoriasis, is associated with greater skin irritation and burning, especially of the surrounding normal skin. Dithranol in a stiffer Lassar's paste base can be confined to the lesions and is preferred by many British dermatologists. Therefore, the usefulness of 'minutes' and a 'short-contact' regime using the standard dithranol paste (Ingram, 1953) was compared with the standard application for 24 hours in two separate studies.

METHODS

(a) Twenty-one consecutive patients with symmetrical plaque psoriasis were randomly allocated to receive treatment by the standard Ingram regime on one arm and leg and the 'minutes' regime (30 minutes) on the other.

(b) Twelve consecutive patients, also with symmetrical plaque psoriasis, were similarly allocated to receive treatment by the standard Ingram regime or the short-contact therapy (2 hours).

In both studies (Table 1) the patients had daily tar baths and gradually increasing but identical doses of UV-B radiation to both sides. The initial dithranol concentration on both sides was 0·5% and was increased to 1, 2 and 3% on alternate days according to tolerance. The

TABLE 1. The three regimes studied

Ingram regime	Short-contact	'Minutes'
Daily tar bath	+	+
Ultraviolet radiation	+	+
Dithranol in hard Lassar's paste	+	+
Stockinette dressings	+	−
Removal of paste after 24 h with arachis oil	Removal of paste after 2 h with arachis oil	Removal of paste after 30 min with arachis oil

side treated with the short-contact or the standard regime was dusted with talcum powder and covered with stockinette dressings for 2 or 24 hours respectively. Any remaining dithranol paste was removed with arachis oil at the end of the treatment period.

Assessment was performed at day 0, 5, 10, 15 and 20 (study a) or at day 0, 7, 14 and 21 (study b). The PASI (Psoriasis Area Severity Index) system of grading psoriasis (Frederickson & Petterson, 1978) was not employed, as assessment of scalp was excluded in both the studies and the area had been found to be the most difficult to assess clinically and the slowest to show improvement (Statham, Ryatt & Rowell, 1984). Instead, erythema (E), palpability (P) and scaling (S) were assessed on a 0–4 scale and the sum of the severity scores (E + P + S) was used to monitor the progress in both the studies. The irritancy and staining were assessed on a 0–4 scale in only one study (study a). Assessment at each visit was performed by the same observer but

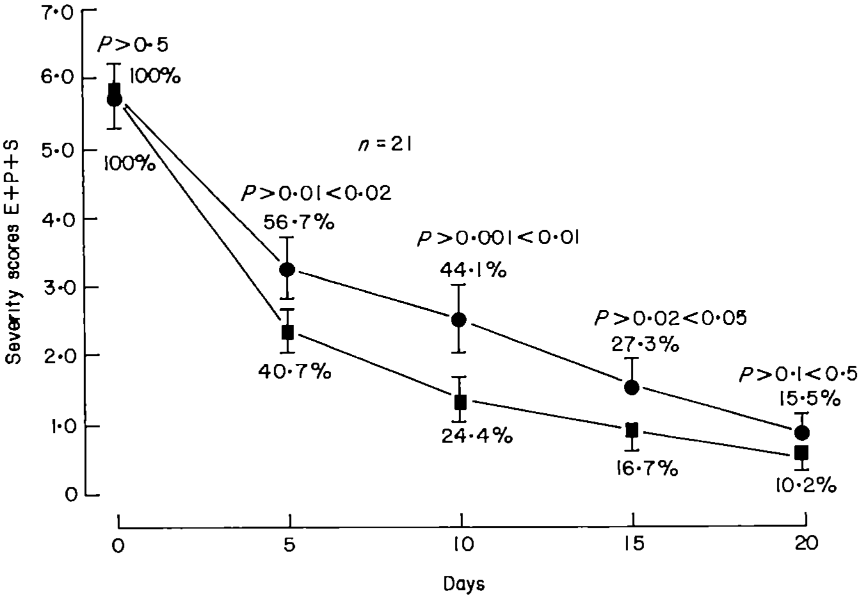

FIGURE 1. Mean severity scores (E + P + S) and percentage reductions (compared with the initial score) at each assessment in 'minutes' vs. standard Ingram regime. (●) Dithranol 30 min. (■) Dithranol 24 h.

assessment scores before and during the trials for both observers (KSR and BNS) were reproducible and comparable. Statistical analysis was with paired t-test and the signed rank test.

RESULTS

All patients completed the trials. Figure 1 shows the sum of severity scores (mean ± s.e.m.) and percentage reduction at each assessment for the 'minutes' vs. standard Ingram regime. Both sides were comparable in severity of psoriasis, as there was no significant difference between the initial severity scores. Significant reduction in scores was seen with both regimes but the standard regime was more effective at day 5 ($P < 0{\cdot}02$), day 10 ($P < 0{\cdot}01$) and day 15 ($P < 0{\cdot}05$). These differences were not maintained subsequently at day 20.

No significant difference was seen at any stage between the 'short-contact' (2 hours) and the standard Ingram regime study (Fig. 2).

The irritant effect over the adjacent uninvolved skin was not significantly different at any stage (study a, Fig. 3).

The degree of staining, not surprisingly, was significantly greater ($P < 0{\cdot}001$) with the standard regime (Fig. 4).

DISCUSSION

The results clearly demonstrate the efficacy of both the 'minutes' and 'short-contact' regimes in clearing psoriasis. However, the '2 hour' regime was more effective than the 'minutes' regime and as good as the standard Ingram regime of 24-hour application.

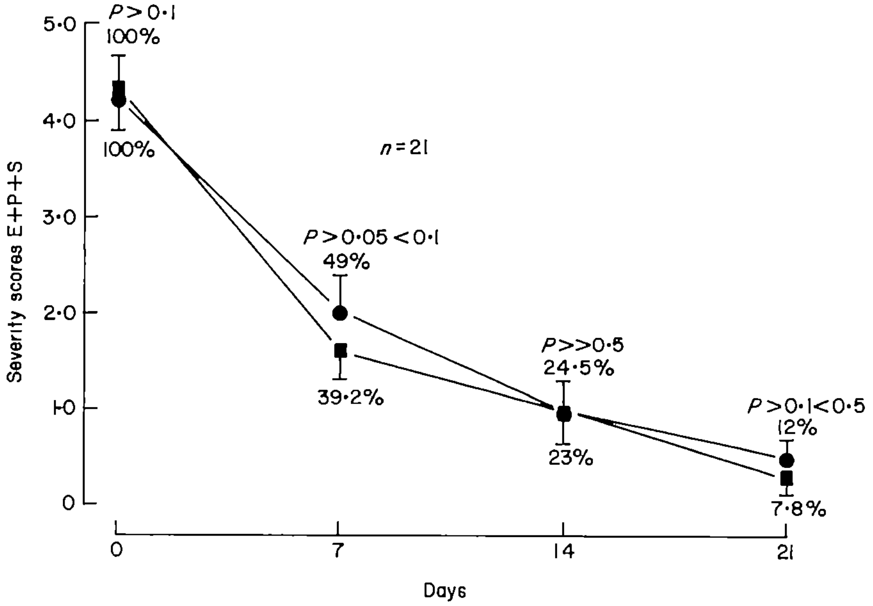

FIGURE 2. Mean severity scores (E + P + S) and percentage reductions (compared with the initial score) at each assessment in 'short contact' vs. standard Ingram regime. (●) Dithranol 2 h. (■) Dithranol 24 h.

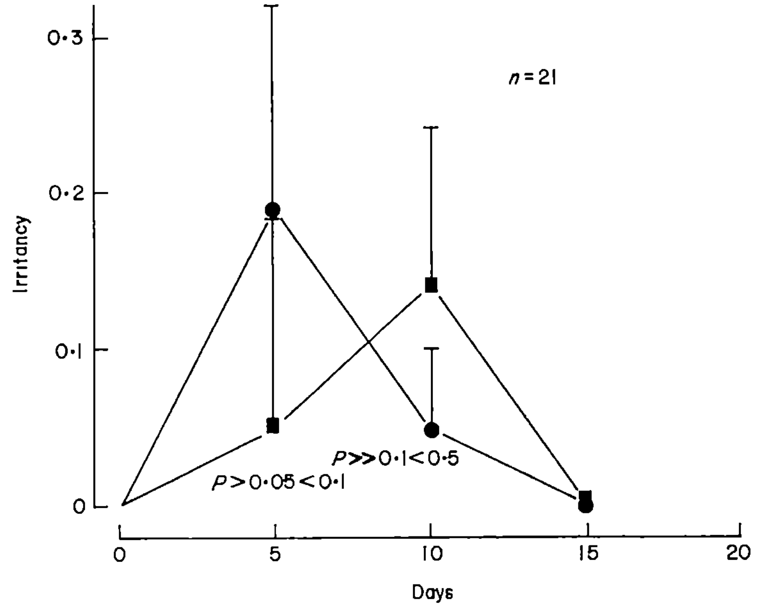

FIGURE 3. Irritancy scores (mean ± s.e.m.) (Study a). (●) Dithranol 30 min (■) Dithranol 24 h.

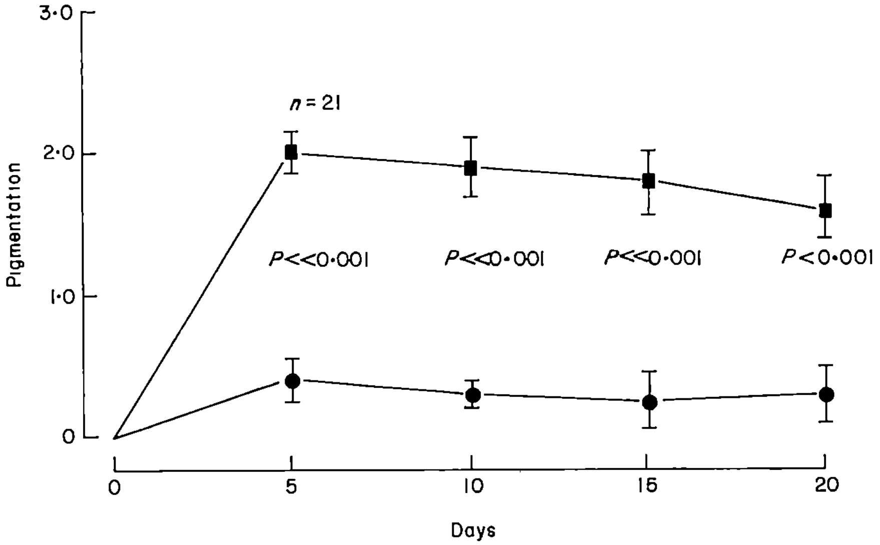

FIGURE 4. Pigmentation scores (mean ± s.e.m.) (Study a) (●) Dithranol 30 min. (■) Dithranol 24 h.

We have shown that it is possible to achieve the full potential of the Ingram regime using an application of dithranol paste for only 2 hours. This has the added advantage of causing less staining than the 24-hour standard Ingram regime and less irritation than the short-duration regimes using YSP/EO based preparations. Dithranol paste is easily removed with arachis oil, and as modified short-contact therapy is found to be useful in well-motivated patients with uncomplicated psoriasis (Donaldson & Cunliffe, 1983), concentrations of dithranol up to 0·5% in hard Lassar's paste for 1 to 2 hours can be recommended for home use with little reservation. Higher concentrations with more co-operative and intelligent patients have also been tried and proved successful. Treatment with the short-contact regime should reduce out-patient attendances and time off work, save valuable nursing time and allow a more normal social life.

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Topical zinc oxide treatment improves arterial and venous leg ulcers

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SUMMARY

We performed a randomized, double-blind study of the efficacy of locally applied zinc oxide on the healing of leg ulcers. Thirty-seven geriatric patients, nineteen with arterial and eighteen with venous leg ulcers, were treated either with a gauze compress medicated with zinc oxide (400 $\mu g\ ZnO/cm^2$) or with an identical compress without zinc oxide. The treatment was assessed from ulcer size measurements and the presence or absence of granulation and ulcer debridement over a period of 8 weeks. The zinc-treated patients (83% success rate) responded significantly better ($P < 0{\cdot}05$) than the placebo-treated patients (42% success rate). The results suggest that healing of leg ulcers is improved after the addition of zinc oxide to the local regimen.

Leg ulcers are commonly due to arterial or venous insufficiency or a combination of these factors. Conservative treatment is used in most cases to treat the disorder (Kitahama *et al.*, 1982). Many topical agents have been tried (Lawrence, 1982), but unambiguous clinical evidence of their efficacy is lacking as a result of difficulties in conducting proper clinical trials (*Lancet* editorial, 1982; Lawrence, 1982).

Zinc used topically on wounds is a folk remedy believed to promote healing; Thomas Hunt (1980) asked 'Is it possible that my mother, who put zinc oxide on all my wounds, was doing me a favour after all?'. Experimental studies in rats have so far failed to verify its effect on open granulating wounds (Norman, Rahmat & Smith, 1975; Williams *et al.*, 1979), although it is absorbed into the blood (Hallmans, 1978; Williams *et al.*, 1979). Wound repair is slow in animals with low zinc levels, but improved after zinc supplementation (Levenson, Seiffer & Van Winkle, 1979). Oral zinc sulphate therapy enhances healing of arterial and venous leg ulcers in patients with a low serum zinc content (Hæger & Lanner, 1974; Hallböök & Lanner, 1972). Zinc given systemically must be continued for 1 to 2 months before clinical effects can be seen (Hallböök & Lanner, 1972).

We have carried out a randomized, double-blind controlled trial to see whether topically applied zinc oxide improves healing of leg ulcers.

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METHODS

Thirty-seven patients with leg ulcers, twenty-four hospitalized and thirteen out-patients, were treated in the order they came to our clinic. Nineteen of the patients (ten females) had arterial and eighteen (fourteen females) had venous ulceration. Eighteen patients (fourteen females) aged 66 to 90 years (median 78 years) were treated with topical zinc oxide and nineteen patients (ten females) aged 68 to 95 years (median 78 years) were treated with placebo. In the zinc-treated group five were out-patients while there were eight in the control group. Six patients in both the experimental and control group had diabetes mellitus.

Criteria for inclusion and exclusion

Ulcers studied were within the limits of 0·5 to 100 cm^2 in area. The ulcers could either be covered with slough or show visible granulation tissue. Patients with two or more ulcers were included if their ulcers were of the same aetiology, and in these cases the largest ulcer was monitored. Diagnosis of ulcer type was made from clinical signs (Knudsen *et al.*, 1981) and by the Doppler technique (Hallböök, 1977). Patients with pressure ratios between ankle and arm of $\leqslant 0{\cdot}5$ were classified as having arterial insufficiency and those with pressure ratios of $\geqslant 0{\cdot}9$ were classified as venous insufficiency. The group with ratios between 0·5 and 0·9 was classified on the basis of clinical signs and symptoms (Knudsen *et al.*, 1981). Patients whose symptoms clearly indicated more than one cause for the ulceration were excluded. Patients receiving systemic zinc or antibiotic therapy were not included.

Treatment

Patients were matched in pairs. The matching variables were time of admission and type of ulcer. The effect of these variables was therefore controlled. To avoid complicated practical matching procedures no other variables were controlled. The treatment was assumed to have the same kind of effect on arterial and venous leg ulcers. Thus the hypothesis that the treatment lacks effect could be tested for a population including both types of ulcers.

The allocation of treatment was made by randomization. One member of the pair was treated with a sterile dry cotton compress impregnated with zinc oxide (400 μg ZnO/cm^2) and the other with a similar compress without zinc oxide. The two dressings could not be visually distinguished from each other.

The ulcers were cleansed with sterile physiological saline solution and dressed once a day in a standardized manner by the regular nursing staff. The ulcers were not surgically debrided. Samples for bacteriological culture were taken in cases with clincal signs of infection in the ulcer area necessitating treatment with antibiotics. Patients suffering from venous insufficiency received compression bandaging and they were instructed to keep their diseased leg elevated whenever possible.

Healing assessment

At the start of the treatment and weekly thereafter the ulcers were inspected, photographed and the outline of the ulcer was traced on to sterile plastic foil. The ulcer area was determined with a planimeter by the same investigator (H.-E.S.) throughout the trial. Patients were observed for 8 weeks or until the ulcer had healed if this occurred sooner. The clinical result was evaluated by a physician not directly associated with the project and the measurements of the ulcer areas and photographs were examined by a surgeon from another hospital. In cases where the opinions of the two doctors differed the measurements and photographs were decisive.

The result was assesed as 'successful' if: (1) visible granulations appeared in the ulcer, or if there were visible granulations present before treatment, the initial ulcer area was reduced by at least 25% in the case of arterial and 50% in the case of the venous ulcers; and (2) the ulcer was free of slough.

The result was assessed as 'unsuccessful' if an enlargement of the initial ulcer area exceeding 50% occurred, antibiotics were required, or criteria (1) and (2) were not fulfilled.

We noted whether the ulcers were healed or not after 12 weeks in the patients who completed the assigned treatment.

Serum analyses

Measurement of the serum zinc concentration (under fasting conditions) was performed on each patient at the end of the trial by a laboratory specializing in trace element analysis (Hallmans, 1978). The albumin concentration was determined by the hospital's laboratory (bromcresol green dye-binding technique; 630 nm).

Statistics

The statistical test was performed at the 5% level, which means that the chance the concomitant variables (like age or initial ulcer area) or other random variables would cause a false significance is less than 5%.

We tested whether the probability of the patient being assessed as 'successful' was the same for the zinc and the placebo group. For this test the result was expressed as 'successful' or 'unsuccessful' as defined above. The statistical test was designed to have the power of 0·95 to detect a 75% success rate in one group and a 25% success rate in the other. The number of patients needed with a conventional test (McNemar's test) to achieve this power was too great to be practicable. Thus a sequential test procedure (Armitage, 1975) was used to minimize the expected sample size. The conclusions are valid for populations of the same kind as the one studied.

RESULTS

The investigation was continued until a significant or a non-significant difference between the two treatments was obtained. This occurred when the line representing a statistically significant ($P < 0{\cdot}05$) advantage of zinc over placebo was intersected (Fig. 1).

Fifteen (83%) of the patients treated with local zinc oxide improved as opposed to eight (42%) of the patients receiving plain gauze compresses. The result of treatment did not seem to correlate with the age of our patients aged 66 to 95 years (Fig. 2). The effect of other possible disturbing variables is described in Table 1. It appears that these factors did not distort the result in either direction.

The local treatment was regarded as 'unsuccessful' in two patients in the zinc group, in one because the ulcer area increased more than 50% and the other after 5 weeks, because of a gradually deteriorating ulcer. This patient was found to have a generalized malignancy. In the placebo group six patients were judged 'unsuccessful'. In three of these the ulcer area exceeded the 50% increase limit and in six the treatment was discontinued after developing ulcer infections. In another patient treated with placebo, the compresses adhered to the ulcer so firmly that the test could not be continued for the full time.

The ulcers in both groups were at about the same stage of healing (Table 2, granulations at start). The median ulcer area was 4·2 cm^2 before and 2·7 cm^2 after the placebo treatment. The

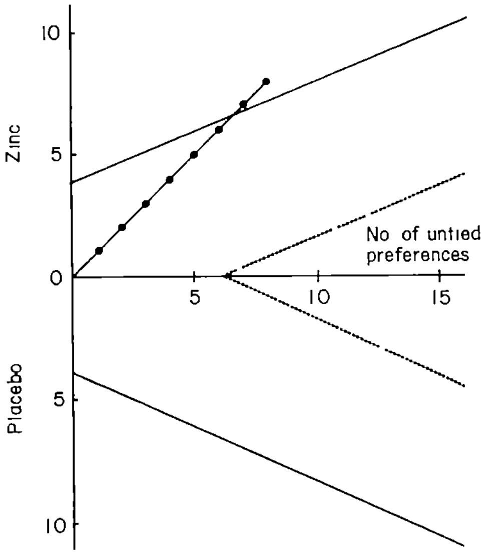

FIGURE 1. Plan and result of the sequential analysis. When one zinc-treated member of a pair was assessed as 'successful' a mark was plotted in the diagram one step to the right and obliquely upwards If a placebo-treated member was assessed as 'successful' the mark was plotted one step to the right and obliquely downwards. If the plot crosses one of the solid borders (as in the present case) a significant difference (at the 5% level) is established. If the plot crosses one of the broken lines, the null hypothesis cannot be rejected.

corresponding values for the zinc group were 3·6 and 0·4 cm^2 (Table 2). In Fig. 3 only the patients whose ulcers healed or who completed the 8-week period are included since this graph illustrates re-epithelialization over a period of time. The healing rates were about 40% during the first month for both groups. After 8 weeks the zinc-treated ulcers had decreased to 9% of their original size (Fig. 3). After 12 weeks of treatment a total of eleven ulcers treated with zinc oxide healed compared with four healed by treatment with placebo (Table 3).

By chance the two largest ulcers were allocated to the placebo group and the three smallest to the zinc group. Changes in ulcer area were expressed in terms of percentage of initial ulcer area. Each ulcer was arranged in order according to its initial area and this value was compared with the total percentage change in area. This analysis yielded no significant trend between the two variables in either group.

The median serum level of zinc was 10·6 μmol/l (range 5·9–14·8 μmol/l) and of albumin 36 g/l (range 28–45 g/l) for the experimental group. The figures for the control group were 10·1 μmol/l (7·7–12·3 μmol/l) and 35 g/l (27–45 g/l) respectively.

One patient in the treatment group and two in the control group complained of pain during dressing of the ulcers. No irritant, allergic or other unfavourable reactions were seen in any patient.

DISCUSSION

Compression therapy should be prescribed for venous leg ulcers and exercise for arterial ulcers (Rook, Wilkinson & Ebling, 1979). The importance of topical applications as a complement to

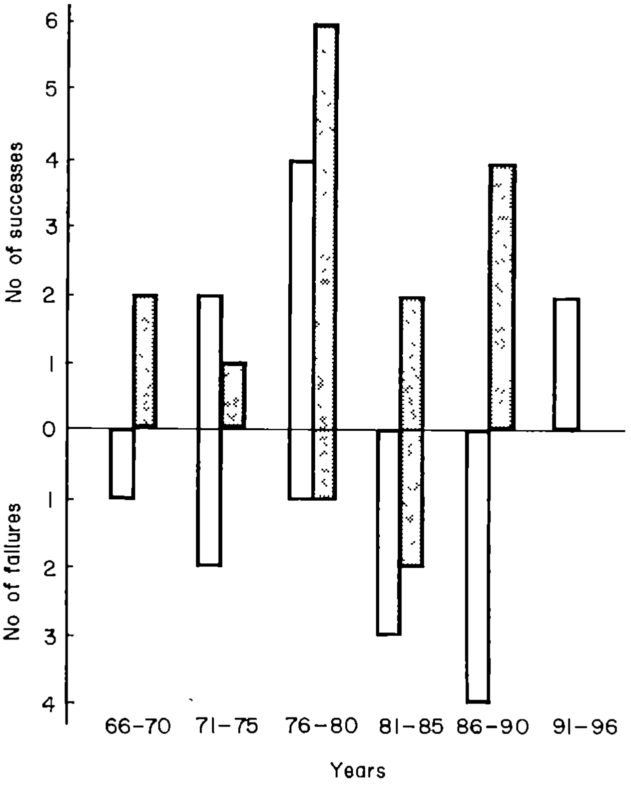

FIGURE 2. Age correlated to the final result. □ Placebo. ▨ Zinc.

TABLE 1. The dependence of patient characteristics on the success rate (no. of successful cases/no. of cases)

	Treatment	
Factor	Placebo ($n=19$)	Zinc ($n=18$)
Sex		
Female	3/10	11/14
Male	5/9	4/4
Diabetes mellitus	3/6	5/6
Ulcer type		
Venous	4/9	8/9
Arterial	4/10	7/9
Ankle/arm pressure ratio (arterial ulcer)		
≤0·35	0/1	3/3
>0·35	4/9	4/6

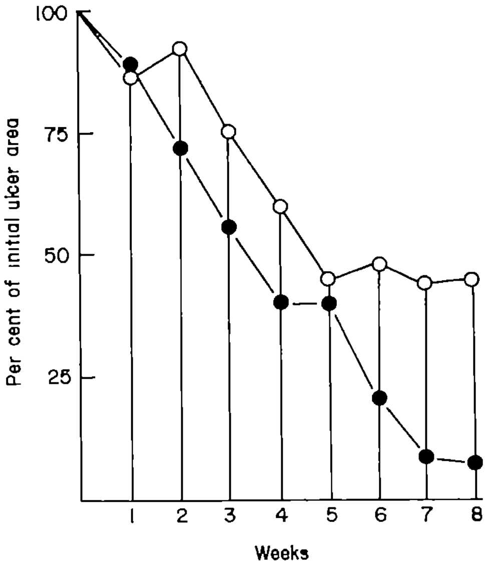

FIGURE 3. Relative ulcer area changes (median values) in percentage of initial ulcer area for the patients completing the 8-week period. (○) Placebo ($n = 12$); (●) zinc ($n = 16$).

TABLE 2. Collected ulcer data from all patients ($n = 37$)

	No. of ulcers			Ulcer area in cm², median (range)	
Treatment	Studied	Granulations at start	Infected during treatment	Initial	Final
Placebo	19	13	6	4 2 (1·4–85·4)	2 7 (0–65·0)
Zinc	18	11	1	3·6 (0·5–14·4)	0·4 (0–17 1)

TABLE 3. Healing frequency

Treatment	Healed within 8 weeks	Healed within 8–12 weeks	Non-healed within 12 weeks
Placebo ($n = 19$)	4	0	15
Zinc ($n = 18$)	6	5	7

the general regimen has been questioned (Rook *et al.*, 1979). In our investigation we used a model where one topical agent, zinc oxide, was compared with placebo as judged from loss of slough, granulation and the rate of re-epithelialization. Zinc oxide application proved to have a superior therapeutic effect.

Arterial ulcers usually require a longer time to heal and are more troublesome to treat than venous ulcers. The similar results obtained for both ulcer types indicate that the venous ulcers diagnosed from classical signs and symptoms might not have had a true venous origin. The presence of arterial insufficiency cannot be excluded, largely due to the advanced age of the patients, although the Doppler measurements failed to detect an arterial disturbance in any patient with a venous ulcer.

Ulcers in patients with diabetes mellitus are often difficult to treat because of atheroma in the large as well as in the small vessels. Neuropathy is also common in these patients. These conditions did not seem to affect the healing process adversely.

No patients in this study showed any general side-effects. Experimentally, Williams *et al.* (1979) were unable to observe any toxic side-effects arising from topically administered zinc oxide in spite of using a hundredfold higher dose than ours. The serum zinc level did not increase in patients on total parenteral nutrition after application of excessive amounts of zinc oxide to an 150 cm^2 area of skin (Derry, McLean & Freeman, 1983).

It is important that topical preparations do not cause any skin reactions since patients with leg ulcers often have sensitized skin (Fräki, Peltonen & Hopsu-Havu, 1979).

Jackson *et al.* (1982) recently found that the zinc concentration in the plasma but not in the skin correlates well with the body zinc status. Measurement of zinc in the blood alone is not a sufficient criterion of a possible zinc deficiency (*Lancet* editorial, 1975). Prasad (1981) claims that whenever elderly patients have wound-healing problems a zinc deficiency should be assumed.

The mechanism of action of zinc in the healing of leg ulcers is unknown. The majority of these ulcers are caused by impaired circulation preventing adequate supply of nutrients to the skin. Burnand *et al.* (1982) reported that the capillaries are often surrounded by fibrin in the venous ulcer-bearing area. In arterial ulcers the blood supply is also reduced. Zinc in the blood therefore has difficulty in reaching the wound site. Local application of zinc should therefore be more efficient than zinc given systemically in promoting wound healing. However, Figure 3 indicates that there is a 5-week delay between the start of local zinc treatment and the appearance of a clear beneficial effect. This observation suggests that the effect of zinc in promoting wound healing is slow during the first month when zinc is either systemically (Hallböök & Lanner, 1972) or locally administered to the wound.

Zinc levels in the blood correlate poorly with the zinc content in the skin (Oon *et al.*, 1974). Biopsies must be taken from the lesion and its surroundings to determine the concentration locally. These procedures may interfere with the healing process and were not performed here.

Apart from being a crucial element in tissue synthesis, zinc has antimicrobial action *in vitro* (Hallmans & Elmros, 1980) and appears to influence inflammatory reactions during wound healing (Tengrup *et al.*, 1980). The lower infection rate in the zinc group could be due to the antibacterial and anti-inflammatory properties of the metal.

Whatever function zinc has during wound healing in human beings, our findings seem to show that topical zinc oxide in the form and dosage used in this investigation is a valuable adjunct in the treatment of leg ulcers.

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Case Reports

Six cases of malignant atrophic papulosis (Degos' disease) occurring in one family

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SUMMARY

A family is described in which a mother and five of her children were affected by malignant atrophic papulosis. This suggests that genetic factors might be involved in this condition.

Malignant atrophic papulosis (MAP) is a rare disorder characterized initially by non-itchy pink or red papules that gradually develop a central area with a white, porcelain-like scale surrounded by a pink ring with telangiectasia. The histopathology shows an endothelial proliferation in the deep dermal vessels and often partial or complete obstruction by a thrombus (Degos, 1979). Similar changes may develop in the vessels of the intestinal wall, especially in the submucosa; cerebral, renal and ocular vessels can also be affected and the disease may be fatal (Degos, 1979). The treatment recommended by Stahl, Thomson and Hou-Jensen (1978) consists of administration of drugs which suppress platelet aggregation.

We now describe six cases in one family.

CASE REPORT

A 40-year-old man presented in 1982 with a rash that had been present for a year and was still spreading. On examination, the trunk, arms and legs showed hundreds of lenticular skin-coloured and pink papules each with a central white porcelain-like scale, surrounded by a pink ring with telangiectasia (Fig. 1). The patient had no local or general complaints. An excision biopsy showed a focal atrophic area in the epidermis with hyperkeratosis. A fibrotic zone was seen in the dermis with an obliterated vessel and proliferation of fibroblast-like cells. Routine immunofluorescence investigation with anti-immunoglobulin antisera showed no abnormalities. Other tests including gastroscopy, sigmoidoscopy, blood tests, in particular immunoglobulins, platelet aggregation and coagulation tests were normal. This clinical and histological picture was compatible with a diagnosis of MAP.

Treatment consisted of aspirin, 500 mg three times daily, because of its action on platelets (Stahl *et al.*, 1978). However, after 1 year of therapy, the lesions were still spreading particularly in the abdominal region, on the back of the hands, the palms and the feet.

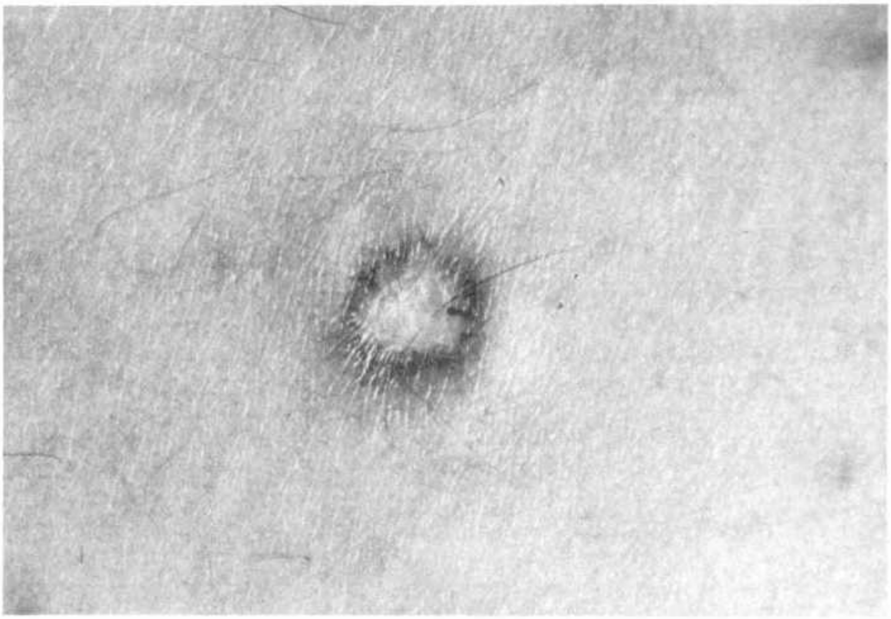

FIGURE 1. Papule with a central porcelain-like scale.

At the suggestion of the patient his first and second degree relatives were examined. Two sisters, two brothers and his mother showed a limited number of lesions as described above. A biopsy from the skin of one sibling showed abnormalities similar to those in the eldest brother. Two other siblings had no local or systemic symptoms (Fig. 2). No consanguinity of the parents was known.

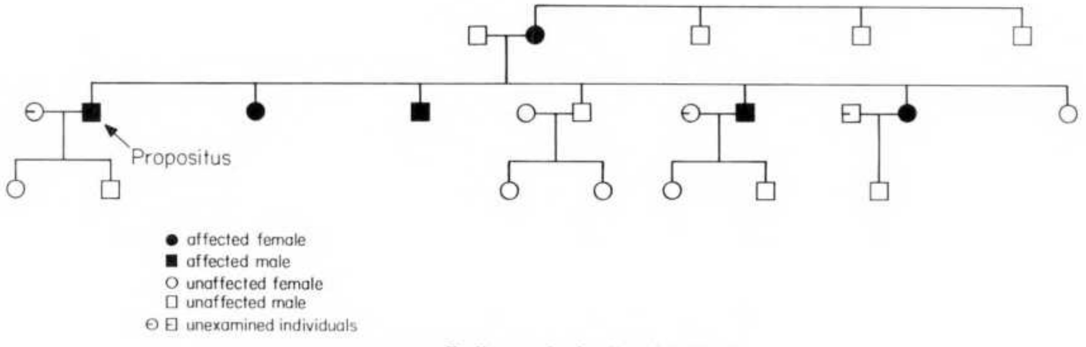

FIGURE 2. Pedigree of a family with MAP.

DISCUSSION

The cases showed the typical eruption of MAP (Degos, Delort & Tricot, 1942). Information concerning the time of onset of the eruption could not be obtained and the mother had reached the age of 58 years without any complaint about her skin, illustrating the mild form of MAP found in the family. The occurrence of this mild form contrasts with the usual course.

Several causes for MAP have been suggested, such as a slow virus infection (Nishida & Howard, 1968), impairment of normal fibrinolytic activity (Black, Nishioka & Levene, 1973), or auto-immune mechanisms (Olmos & Laugier, 1977). The occurrence of MAP in a mother and child has been reported by Hall-Smith (1969). The occurrence of the disease in six members of one family suggests a genetic aetiology. Black (1976) found a male: female ratio of about 3 : 1 and Metz, Amschler & Henke (1980) mention a male predominance (1·6:1). In our patients, however, no male predominance was observed. Our findings would be compatible with an autosomal dominant inheritance.

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Comment

LICHEN PLANUS AND LIVER DISEASE

In recent years, a number of case reports have drawn attention to the possible association of lichen planus (LP) with chronic liver disease (Seehafer *et al.*, 1981; Powell & Roy, 1981; Rebora, 1981; Monk & Pembroke, 1981; Colandia, 1972; Graham-Brown, Sarkany & Sherlock, 1982). The association remains controversial. Some authors recommend careful screening of patients with LP for underlying liver disease, whilst others consider the association rare, or due to chance. The coexistence of LP and 'autoimmune' liver disease has also been used to support the hypothesis that LP is caused by a primary immunological disturbance (Rebora & Rongioletti, 1984). Of four large studies expressly concerned with the possible association between lichen planus and liver disease, two have drawn their study populations from dermatology clinics, whilst the other two emanate from specialist hepatology units.

LIVER DISEASE IN PATIENTS WITH LICHEN PLANUS

Stimulated by case reports of LP and liver disease (Rebora, 1981; Monk & Pembroke, 1981) Rebora and Rongioletti (1984) undertook a retrospective study of ninety-five patients with LP who had been managed over a 26-year period in their dermatology unit. The investigators successfully contacted forty-four patients willing to be screened for liver disease. Of the remainder, sixteen had died, and thirty-five failed to respond to their follow-up request. Serum bilirubin was normal in all forty-four patients, but six had abnormal liver enzyme profiles. Antinuclear (ANA) and/or smooth muscle (SMA) antibodies were detected at low titre in four of the six patients, and although all were HBsAg negative, three had serological markers of previous hepatitis B infection. Liver biopsy was performed in all six patients and in five the histology was compatible with chronic active hepatitis (CAH). The sixth patient had steatosis. Of the sixteen patients who had died, one was known to have CAH, and another died in hepatic failure. No further details were available on this group. The authors draw two major conclusions: (i) CAH occurs in 11·3% of LP patients and this exceeds the expected prevalence of cirrhosis in their geographical area; (ii) the association of LP with an autoimmune disease (CAH) supports the hypothesis that LP is caused by a primary aberration of the immune system.

This retrospective study has many faults. The LP population was comprised of a highly selected group of forty-four patients drawn from a potential population of ninety-five. The exclusion of fifty-one patients from the analysis introduces serious bias in the data, and probably overestimates the true prevalence of liver disease. In addition, although the prevalence of liver disease was higher than expected in the general population, the authors failed to make the critical comparison with age- and sex-matched controls affected by other skin disease. In the absence of control data, the study cannot reliably confirm an increased prevalence of liver disease in LP.

The authors also speculate that the association of LP with CAH supports the concept of an immunogenic basis to LP. However, CAH is purely a descriptive term used by pathologists to describe portal inflammation and piecemeal necrosis of the liver. This histological pattern is characteristic of autoimmune (lupoid) hepatitis, but is also commonly observed in chronic type B and non-A/non-B hepatitis, alcohol-related liver disease, drug hepatitis and Wilson's disease. The five patients with CAH do not conform with a diagnosis of 'lupoid' CAH. This disease occurs mainly in women, and presents with florid signs of liver disease. The patients are usually jaundiced, and transaminase levels are markedly increased. There are no specific serological markers of the disease, although the majority of patients have markedly elevated IgG levels and high titres of antinuclear and smooth muscle antibodies. Four of the six patients reported by Rebora were male, and none was jaundiced. Autoantibodies were present in low titre (a non-specific finding in many forms of liver disease), and in three of the five patients with CAH past infection with hepatitis B might explain the liver pathology. Neither alcohol nor other causes of CAH were rigorously excluded. Contrary to the authors' conclusions, it cannot be inferred from this study that LP is significantly associated with autoimmune CAH.

In a prospective study, Mobacken *et al.* (1984) studied the prevalence of liver disease in fifty-four patients referred to their unit because of severe and refractory oral LP. Two patients were known to have chronic liver disease (one cryptogenic cirrhosis, one primary biliary cirrhosis). A further four patients had minor abnormalities of routine liver function tests, but in none was the abnormality severe enough to justify liver biopsy. Low titre ANA and SMA were observed in seven and three cases respectively, and the single patient with primary biliary cirrhosis (PBC) had a positive anti-mitochondrial antibody (AMA). The authors conclude that liver disease is rare in LP, and is probably coincidental rather than related. This prospective study was highly selected, as it was confined to patients with severe oral LP. There is at present no prospective controlled study designed to determine the prevalence of liver disease in an unselected group of LP patients.

LICHEN PLANUS IN PATIENTS WITH LIVER DISEASE

In patients with primary biliary cirrhosis (PBC), a clearer association with LP has been established. Two liver centres conducting controlled trials of penicillamine treatment in PBC have recognized an association between the two disease. Seehafer *et al.* (1981) from the Mayo Clinic described six PBC patients who developed LP 4 to 16 months after starting treatment with pencillamine. In four of six patients, the LP resolved on withdrawal of treatment, and in three patients, repeat challenge caused recurrence. The authors noted that LP has been rarely reported with pencillamine, and suggested a special susceptibility in PBC. The lack of case reports might, however, be due to under-reporting, as penicilliamine-related mucocutaneous reactions are common, but are rarely biopsied since they rapidly resolve on stopping treatment. The possible association between PBC and LP was strengthened by a report from the Royal Free Hospital, describing five patients with both diseases (Graham-Brown, Sarkany & Sherlock, 1982). Unlike the previous study, only one patient had received pencillamine. The authors noted that lichenoid skin lesions are characteristic of chronic graft-versus-host disease (Saurat & Gluckman, 1977), and that PBC has many other features of this iatrogenic disease (Epstein, Thomas & Sherlock, 1980). They suggest that the predisposition to LP in PBC reflects an underlying graft-versus-host-like syndrome occurring in PBC.

In a follow-up report, the Mayo Clinic group extended their observation reporting twenty-four PBC patients with LP, seven of whom were not treated with pencillamine (Powell, Rogers & Dickson, 1982). This confirmed the Royal Free observation that LP occurs spontaneously in PBC, but the authors stressed the unusual predisposition in pencillamine-treated patients.

CONCLUSION

The studies are imperfect but provide the impression that in dermatology units the association of LP with liver disease is unusual and probably coincidental. In patients with LP the search for liver disease is likely to be unrewarding, but routine liver tests should be performed at presentation. Suggestive findings of PBC include an increased alkaline phosphatase and the diagnosis should be strongly suspected if the AMA test is positive.

In liver units LP occurs almost exclusively in PBC patients, especially those treated with pencillamine. The association is interesting because features of both LP and PBC occur in chronic graft-versus-host disease, and it is possible that penicillamine treatment unmasks an inherent predisposition to LP in PBC.

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Society Proceedings

Proceedings of the 230th Meeting of the Netherlands Society of Dermatology and Venereology, Utrecht, 4 February 1984

PRESIDENT: PROFESSOR DR E. VAN DIJK
HOST: PROFESSOR DR E. YOUNG

SUMMARIES OF PAPERS

Pachyonychia congenita syndrome: electron microscopic, histological and clinical findings of an unusual case

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The Pachyonychia congenita syndrome (PCS) is an ectodermal dysplasia of which the striking thickness of all nails is the most constant symptom. The syndrome is transmitted by an autosomal dominant gene with high penetrancy and a variable expression (Hodes & Norins, 1977). Various symptoms can be present and a clinical subclassification in three types can be made (Schönfeld, 1980). This patient, whose electron

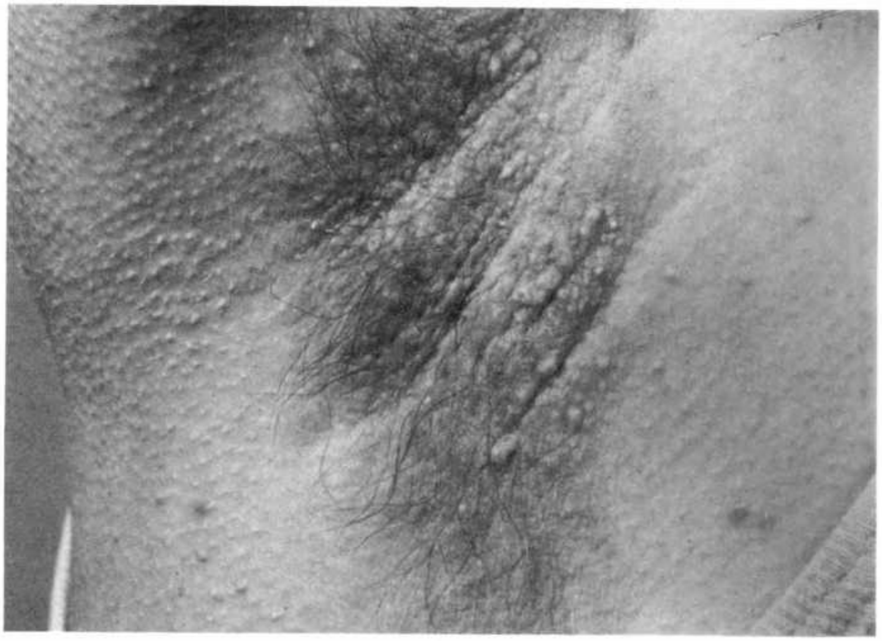

FIGURE 1. Pachyonychia congenita. The unusual papillomatous lesions in the axilla.

microscopic, histological and clinical findings are presented, is an unusual case because of the severity of her symptoms and the additional lesions in the axillae and the vulvar area.

A woman, aged 22 years, developed at birth the usual symptoms of PCS type I: thickening of all nails, palmo-plantar keratosis with blister formation under and around the callosities of the feet, follicular, filiform keratoses all over the body, oral leucokeratosis and hoarseness. No steatocytoma multiplex, dental or hair abnormalities were found. At the age of 12 years she developed symptoms in the axillae, the corners of the mouth, the vulvar and peri-vulvar region, all areas which are easily macerated. The axillary folds showed a palm-sized, light brown, irregularly papillomatous, somewhat keratotic area; the central parts were pale pink and slightly macerated (Fig. 1). The lesions in the corners of the mouth and in the vulvar and peri-vulvar region were very similar to those in the axillae. The patient was the only member of her family affected. Most of the time she was confined to a wheelchair owing to the pain caused by the enormous callosities and blisters. Specially made shoes increased her walking radius from 25 metres to more than 100 metres without too much discomfort.

A biopsy from the axillary fold revealed irregular acanthosis with a papillomatous surface, marked

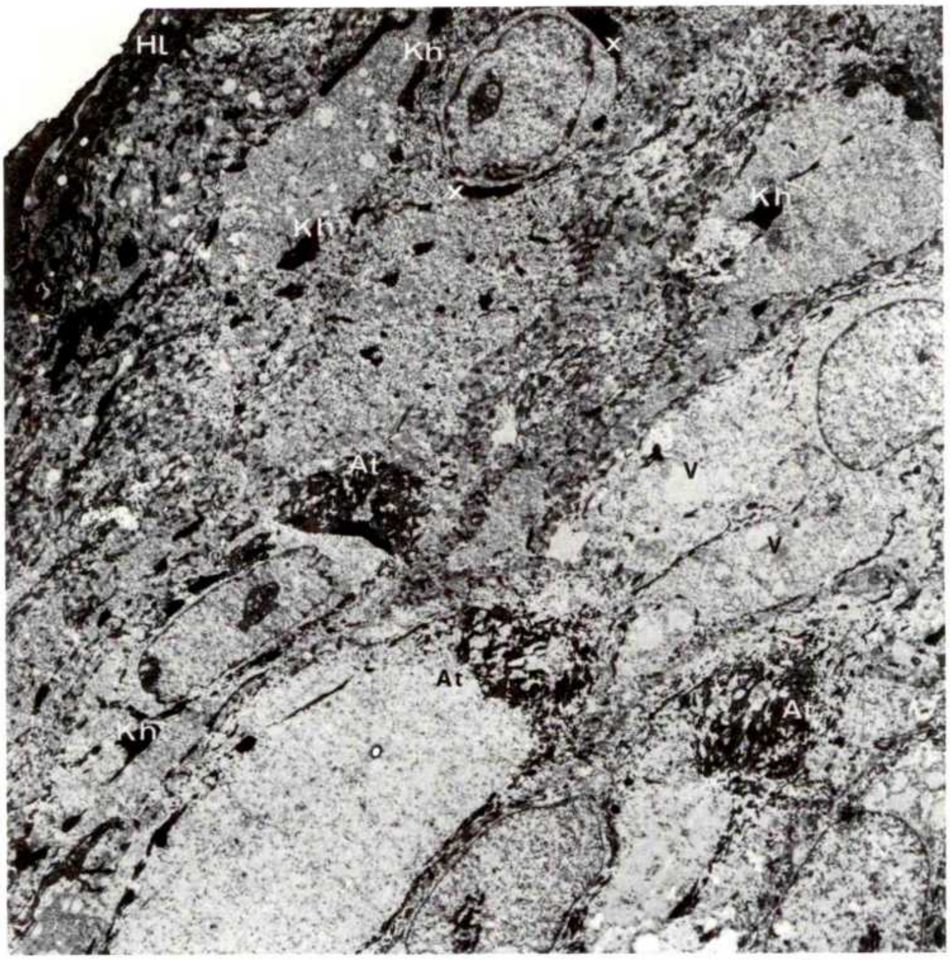

FIGURE 2. Horny layer (HL) and granular layer showing keratinocytes with aggregates of tonofibrils (At), keratohyalin granules (Kh), some in perinuclear arrangement (X), and the initial stage of vacuolization (V).

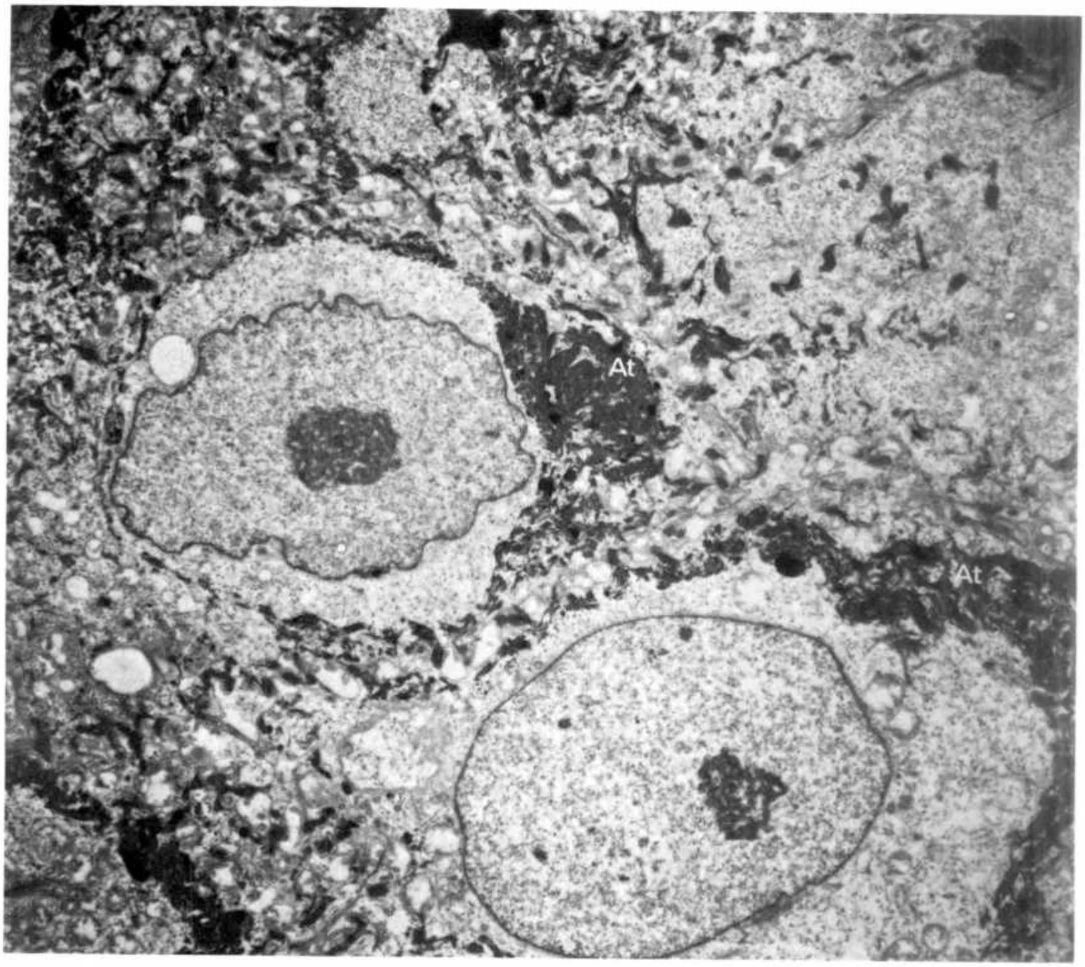

FIGURE 3. Higher magnification of keratinocytes with aggregates of tonofibrils (At).

hyperkeratosis with small para-keratotic foci and follicular plugging. The stratum granulosum was alternately thickened, narrowed or almost absent. There was hypergranulosis. The cells in the upper layers of the stratum Malpighii showed focal intracellular oedema and vacuolization. In the dermis no significant changes were found. These findings were identical to the pathological changes in other lesions of the PCS (Schönfeld, 1980).

Electronmicroscopy of the lesion in the axilla showed abnormal keratinization and the initial stage of bulla formation in the stratum Malpighii. A few layers of dense horny cells covered the outer surface of the epidermis. Underneath there were keratohyalin granules in different sizes, keratinosomes and perinuclear vacuolization of the keratinocytes. Many keratohyalin granules showed a perinuclear arrangement. The association of tonofibrils and desmosomes was largely disturbed. The most striking feature was the formation of aggregates of thick bundles of tonofibrils, all situated without exception on the same lateral side of the keratinocytes (Figs 2 and 3). The bullae were characterized by perinuclear vacuolization of the keratinocytes, increasing during their migration to the surface. As far as we know, aggregates of thick bundles of tonofibrils on the same side of the keratinocytes have not previously been described in the literature (Perrot, Schmitt & Thivolet, 1973; Thormann & Kobayasi, 1977).

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Carotenoids as therapeutic agents

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Carotenoids are pigments which are to a great extent responsible for the orange-red colour of numerous vegetables, fruits, birds and fishes. In photosynthesis they act as supplementary light receptors next to chlorophyll. Furthermore, some carotenoids are precursors of Vitamin A in animals and they are capable of protecting cells and tissues from the harmful effects of visible light (photoprotection).

Mutants of bacteria and algae which lack carotene do not survive exposure to visible light, whereas specimens which contain the pigment develop normally. The photoprotective properties have been used in the therapy of photodermatitis of porphyric origin. Since β-carotene proved to be photoprotective in mice which had been made photosensitive by injections with haematoporphyrin, it was also administered to patients with erythropoietic protoporphyria (EPP). This is a disturbance of human haem synthesis in which protoporphyrin accumulates in the body, in particular in erythrocytes. Here protoporphyrin acts as photosensitizer; the photodermatitis is induced by visible light, especially violet (405 nm). In moderate cases a distinct increase of the tolerance to sunlight may be achieved with β-carotene. However, in very sensitive patients where the symptoms are provoked even by 5–10 minutes' exposure to sunlight, the results are disappointing. In the author's opinion carotenoid therapy in other photodermatoses, e.g. polymorphic light eruption, is not very successful.

EPP photodermatitis is probably initiated by the formation of oxygen radicals, notably singlet oxygen. The photoprotective effects of carotenoids may be based on their capacity to quench the radical substances. Consequently they might be tried as therapeutic agents in non-photogenic processes where singlet oxygen plays a role.

Chenodeoxycholic acid treatment in erythrohepatic protoporphyria

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Erythrohepatic protoporphyria (EPP) is characterized by an excessive production of protoporphyrin (PP) in the bone marrow and liver. Patients with EPP are photosensitive, often have gallstones and may develop a cirrhosis with PP deposits in the hepatocytes and bile canaliculi. PP is excreted only via the bile; this excretion is bile salt dependent.

The aim of the study was to investigate the effect of chenodeoxycholic acid (CDCA) administration on the excretion of PP in patients with EPP. Five patient with EPP were studied. Faeces were collected for 7 days, and 10 ml of bile was sampled daily by means of a duodenal tube. CDCA was given in a dosage of 15 mg/kg/day from the 8th day. The faeces and bile collections were continued for a further 10 days. Blood was taken every 2 to 3 days. PP was measured in the bile, faeces and erythrocytes by high pressure liquid chromatography (HPLC) and fluorimetry. Bile salts were measured enzymatically.

TABLE 1.

Patient	PP in bile (μmol/mmol BA)		PP in faeces (μmol/g dry weight)		PP in erythrocytes (μmol/l)	
	Before CDCA	After* CDCA	Before CDCA	After* CDCA	Before CDCA	After** CDCA
S	0·52	0·19	0·65	0·06	63·8	48·7
B	1·11	0·50	1·04	0·37	81·7	64·0
G	4·75	1·43	7·30	2·27	61·7	46·7
Z	—	—	2·77	0·75	74·0	35·0
J	5·98	3·20	7·10	3·89	63·5	54·4

$^{*}P < 0{\cdot}01$ and $^{**}P < 0{\cdot}05$, Wilcoxon test mean values given.
BA = bile acids.

Results. Following the administration of CDCA the concentration of PP in the bile decreased significantly. This was associated with a marked decrease in the excretion of PP in the faeces, and was followed in all patients by a decrease in the erythrocyte PP concentration. A significant linear relationship was found between the biliary concentration of PP and the total PP excretion in the faeces (Table 1).

Conclusion. CDCA treatment caused a marked decrease in the excretion of the PP via faeces in patients with EPP. The subsequent decrease in erythrocyte PP concentrations suggests that CDCA inhibits the production of PP in the liver. These findings suggest an interaction between bile acid metabolites and haem synthesis. This opens new possibilities of influencing porphyrin formation in patients with porphyria.

Clinical experience with tranexamic acid (Cyclocapron®) in urticaria and angio-oedema

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The therapeutic benefit of protease inhibition in urticarial diseases has been neglected. Only a few reports exist in the literature. The results of treatment of chronic urticaria with the kininogenase inhibitor aprotinin (Trasylol®) were given by Juhlin & Michaëlsson (1969) and by Berova, Petkov & Andreev (1974). The beneficial effects of the more specific plasmin inhibitors ε-amino-caproic acid and tranexamic acid (TA) have been reported almost exclusively for hereditary angio-oedema (Champion & Lachmann, 1969; Hadjiyannaki & Lachmann, 1971; Sheffer, Austen & Rosen, 1972). For the treatment of chronic urticaria with TA only one, small, placebo-controlled study is available (Laurberg, 1977). In the seventeen patients selected for their low C_1-esterase inhibitor levels, no significant clinical changes were observed. However our own experience, gained over several years, showed TA to be very useful. In an open clinical trial seventy-four patients were seen with various types of urticaria and treated with TA, 3 × 500 mg/day during 4 weeks. An open trial was chosen as most patients had already experienced several medical treatment periods with other drugs. These adult patients were selected mainly because of an inadequate response to conventional therapy. Patients with an acute attack of angio-oedema were given TA 3 × 1000 mg/day for 5 days, while cyclic urticaria was treated with TA 3 × 500 mg/day for 7 days during 3 cycles. Daily records were kept by the patients. The usual pre- and post-treatment laboratory investigations were instituted. The results of the study are given in Table 2.

The overall figures of the group of patients under investigation are comparable to those of Berova *et al.* (1974) for aprotonin treatment. It is noticeable that TA treatment alone had a marked effect in diseases

TABLE 2. Results of treatment with tranexamic acid in urticaria

	n	Cured	Improved	Equal or worsened
Acute angio-oedema	8	6	0	2
Recurrent angio-oedema	4	2	0	2
Factitious urticaria	7	1	3	3
Cholinergic urticaria	9	2	1	6
Pressure urticaria	6	2	2	2
Delayed pressure urticaria	11	8	1	2
Cold urticaria	5	2	0	3
Chronic idiopathic urticaria	16	5	6	5
Cyclic urticaria	3	1	0	2
Urticaria–vasculitis	5	3	1	1
Totals	74	32(43%)	14(19%)	28(38%)

normally thought to be mediated by histamine pathways. Moreover TA proved to be a very successful inhibitor in selected patients with delayed pressure urticaria and in patients with acute angio-oedema, both groups being refractory to steroids and antihistamines. Some patients with chronic idiopathic urticaria responded remarkably well, taking into account that all of them were non-responsive to antihistamines. The possibility of an even more effective treatment in these patients exists when TA is combined with antihistamines. The rather low dose of TA given in this study had no effect on bleeding times according to Ivy, nor on liver function tests. *In vitro* no measurable activation or inhibition occurred on the complement or kallikrein-kinin system.

Adverse reactions were observed in ten patients (13·5%). They consisted of headache (3), nausea/abdominal pain (3), increase of urticarial activity (2), flushing/perspiration (2), weakness/tiredness (2). These patients were all classified as equal or worsened. In the selection of patients to be treated with TA one should avoid those having an increased risk of thrombosis.

It is concluded that a favourable effect of low-dose treatment with TA in patients with urticarial disorders can be obtained, especially in those who are only partly responsive to antihistamines. Further double-blind studies are needed to confirm this impression.

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Granular IgA deposits in vessel walls of clinically healthy skin

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Granular deposits of IgA occur in the walls of superficial vessels of clinically healthy skin in 75% of patients with primary IgA nephropathy, 100% of those with Henoch–Schönlein purpura (HSP), and 63% of those with alcoholic liver disease (Baart de la Faille-Kuyper *et al.*, 1967; Kater *et al.*, 1979). The aim of this study was to investigate the disease specificity of the presence of IgA.

Retrospectively, 1030 skin biopsies of clinically normal skin, sampled during 1981 and 1982 for routine immunohistochemistry, were investigated for IgA deposits in vessel walls. We excluded biopsies with concomitant immunoglobulin deposits at the dermo-epidermal junction (as found in SLE) or IgA in dermal papillae (as found in DH). One hundred and twenty-three biopsies were found to be positive. The clinical diagnoses are summarized in Table 3.

In a second study concerning only patients with primary renal diseases with exclusion of primary IgA nephropathy, HSP, alcoholic liver disease and systemic diseases, IgA deposits were found in 13 of 101 biopsies (Table 4).

TABLE 3. Clinical diagnosis in 123 patients with IgA-positive vessel walls

Henoch–Schonlein purpura	25	50
Primary IgA nephropathy	9	
Recurrent haematuria (no renal biopsy)	16	
Alcoholic liver disease		10
Various connective tissue disorders		27
Focal infections	8	36
Various renal diseases	13	
Other diseases	12	
No sufficient data	3	
		123

TABLE 4. Clinical diagnoses in 101 patients with renal diseases

IgA-positive vessel walls		
Rapidly progressive glomerulonephritis	1	13
Membranous glomerulonephritis	2	
Mesangio-capillary glomerulonephritis	2	
Diabetes mellitus	1	
Analgesic nephropathy	2	
Alport's disease	5	
IgA-negative vessel walls		
Glomerulonephritis	57	88
Other renal diseases	23	
Alport's disease	8	
		101

Conclusion. IgA deposits in vessel walls of clinically healthy skin can be used as a marker for primary IgA nephropathy, HSP, autoimmune diseases and alcoholic liver disease. In the absence of these diseases, deposits were found in 3% of a large population and in 13% of patients with other renal diseases.

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Improving the effectiveness of phototherapy for psoriasis

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Modification of the spectral composition of the radiation used in phototherapy offers the possibility of improved therapy. Starting from UV-B therapy for psoriasis using fluorescent sunlamps (Philips TL-12), we examined the influence of a shift towards the longer wavelengths. This became feasible by the development of an experimental fluorescent lamp (EFL), emitting mainly in a narrow peak around 311 nm, with a half value width of 3 nm. The TL-12 lamp has a much higher output in the wavelengths around 300 nm.

Comparisons of therapeutic effectiveness were performed in two series of patients, all having widespread psoriasis vulgaris. In one series (eight patients) each patient received a full course of treatment with the EFL-radiation; the results were compared with those in a previous treatment with TL-12 radiation. In the second series, nine patients having symmetrically distributed lesions, received treatment with EFL-radiation on one half of the body and with TL-12 radiation on the other half. In both series, each patient served as his own control. Treatments to be compared were given according to the same protocol; each exposure was aimed at eliciting a slight erythema.

The therapeutic results were assessed by independent observers. Statistical analysis showed that the therapeutic effects achieved with EFL-radiation were better than with TL-12 radiation ($P < 0{\cdot}05$ in both series).

An animal experiment was performed to compare the carcinogenic effects of the two spectral distributions. One group of twenty-four hairless mice was exposed to the full radiation from the TL-12 lamps. A second group of twenty-four mice was exposed to the EFL-radiation. The ratio of the daily suberythemal doses from both sources was in direct proportion to the doses required to induce a slight erythema and oedema in the animals used.

By regular assessments the skin reactions were recorded. All animals in both groups developed skin tumours. The results show that the median tumour induction time was significantly longer ($P < 0{\cdot}05$) in the mice exposed to the EFL-radiation. This holds only for minimal tumours. For larger tumours the median induction time was still longer, but not significantly ($P < 0{\cdot}1$).

A more realistic approach would be to look at the risk of getting tumours in a relatively small percentage of the population. For a figure of 20% of tumour-bearing mice, the tumour induction time for all tumour sizes is significantly longer ($P < 0{\cdot}01$) in mice exposed to the EFL-radiation. In conclusion: the EFL-radiation is more effective in the treatment of psoriatic patients and less carcinogenic in mice.

Tattoo removal by CO_2 laser

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Thirty-nine patients with tattoos were treated with a Coherent 400 CO_2 surgical laser using the method described by Reid and Muller (1980). All patients were treated under local anaesthesia with a 2% lidocaine solution. Two patients developed severe hypertrophic scars and in six other patients there were slight signs of hypertrophic scar formation. In twelve patients some degree of pigmentation was still seen in the atrophic scar. Although the removal of the tattoo was not esthetically successful in every case, almost all patients were very pleased with the result of the dermabrasion with the CO_2 laser. It is concluded therefore that the CO_2 laser is a useful instrument for the removal of tattoos (Figs 4 and 5).

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CASE REPORTS

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Lupus vulgaris

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History. Male, aged 38 years, Moroccan. The patient presented with progressive chronic inflammation of the skin, which began with a primary lesion on his left foot in his youth, and gradually extended upwards, complicated by elephantiasis of the leg. At the age of 16 years scrofuloderma was present. 6 years later, lesions on his trunk and elephantiasis of the scrotum developed.

Examination. Patchy red-brown lesions were present on the trunk, and the scrotum and left leg had keratosis and pale atrophic scarring. Apple-jelly coloured nodules were observed in the margins of many lesions as well as in areas of atrophy. The leg and scrotum showed verrucosis and lymphoedema (elephantiasis) (Fig. 6). In the left axilla a firm lymph-node mass was palpable.

Laboratory tests. X-ray of abdomen showed calcified lymph nodes. X-ray of thorax was normal. Tuberculin test developed 19 mm induration, with a bulla in 72 hours. A culture of a skin biopsy of the trunk grew typical *Mycobacterium tuberculosis*. Further investigations were negative.

Histology. In the dermis a mononuclear granulomatous inflammatory infiltrate with large multi-nucleated cells of the Langhans' type was present (Fig. 7). There was no caseation necrosis. Ziehl–Neelsen staining was negative for acid-fast bacilli.

Treatment. Oral intake of isoniazid 300 mg/day, ethambutol 1200 mg/day and pyrazinamide 1500 mg/day.

Discussion. Lymphoedema (and elephantiasis) is a rare complication of lupus vulgaris, caused by extensive scarring (Wolff, 1971).

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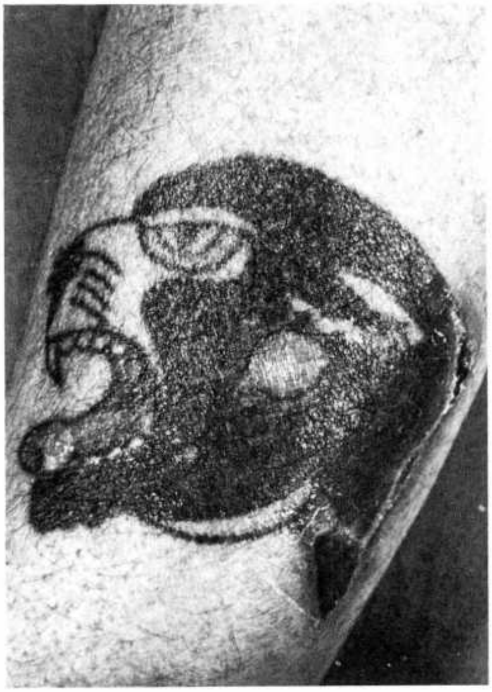

FIGURE 4. Tattoo before laser dermabrasion.

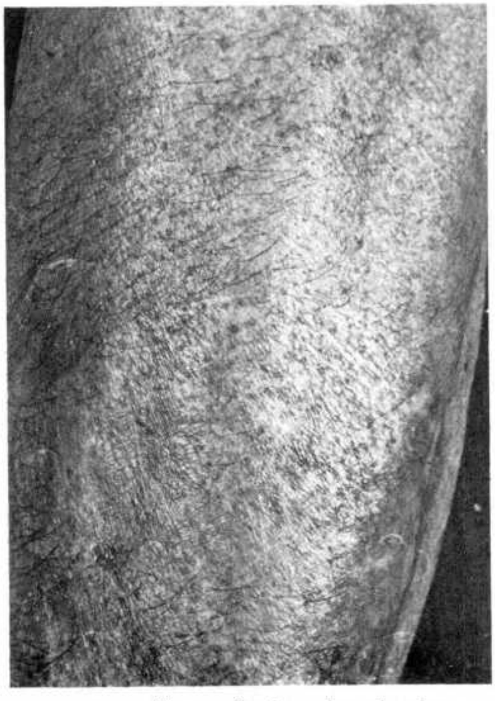

FIGURE 5. Tattoo after laser dermabrasion.

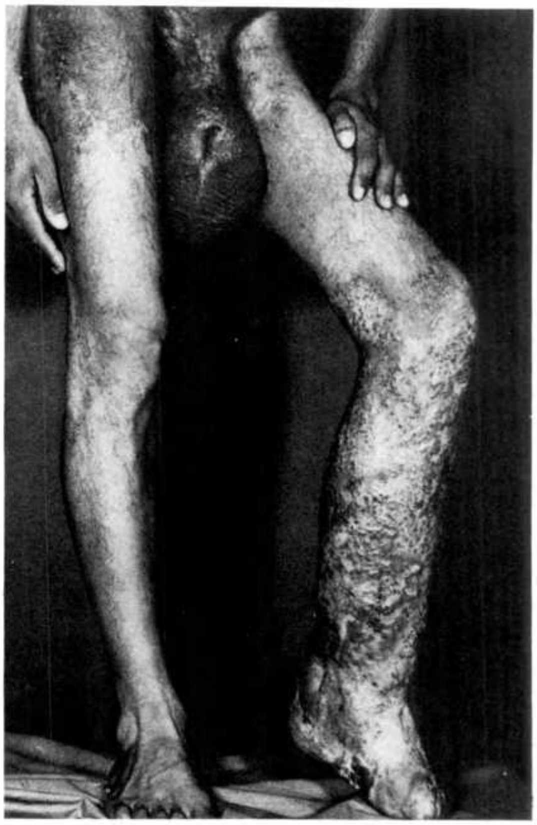

FIGURE 6. Elephantiasis of scrotum and left leg; verrucous lesions on the lower leg and foot.

Transient eosinophilic nodules (Thivolet)

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History. Female, aged 71 years. In January 1981 the patient suddenly developed skin lesions on the trunk, arms and legs with generalized pruritus. A bacterial superinfection of the skin was treated with antibiotics. Although the rash at first disappeared, it reappeared 1 month later. At the time of referral she used no oral or local medication. Her medical history revealed a sero-positive rheumatoid arthritis and a bipolar psychosis.

Examination. An erythemato-papular rash was present on the back, abdomen, buttocks and extremities. Moreover nodules, in part pustular, occurred in asymmetrical distribution on elbows, buttocks and the flexor sides of the knees (Fig. 8). The general condition was fair but there were signs of destructive arthritis.

Laboratory tests. E.S.R.: 40 mm, CRP 1·0 mg%. Total PMN: $6{\cdot}7 \times 10^9$/l, eosinophils 17%, basophils 1%, rod 2%, segment 34%, lymphocytes 30% and monocytes 16%. Total eosinophil count: $1{\cdot}0 \times 10^6$/l. ANA: positive 1:100, rheumatoid factor positive, RA-haemagglutinin: 1:1280. *Staphylococcus aureus* was cultured from the skin, nose, throat, ears and perineum. Further investigations were negative.

Histology. Both rash and nodules showed parakeratosis, acanthosis, sometimes spongiosis, vesiculation

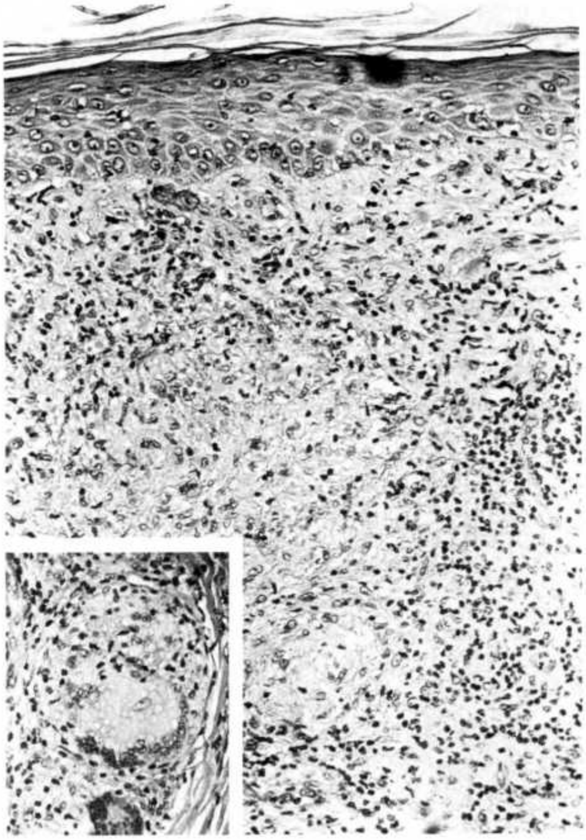

FIGURE 7. Mononuclear granulomatous inflammatory infiltrate with large multi-nucleated cells of the Langhans' type (see detail).

and PMNs infiltrating the epidermis. In the upper and mid-dermis huge infiltrations with eosinopils were observed. The blood vessels were distinct and sparse perivascular concentrations of lymphocytes and histiocytes were noted.

Treatment. The bacterial infection was treated with oral flucloxacillin and local silver–sulphadiazine ointment for 10 days. The rash decreased during this time and subsequently faded completely. Both the nodules and the eosinophilia disappeared spontaneously within the next 4 months. At that time *Staphylococcus aureus* still could be isolated from the nose, throat, ears and perineum. There has been no recurrence of the skin lesions.

Discussion. The differential diagnosis included hypereosinophilic syndrome, Wells' eosinophilic cellulitis, drug eruption or a dermatological manifestation of rheumatoid arthritis. The clinical picture seems to fit best the transient eosinophilic nodules, described by Thivolet *et al.* (1980). This disease heals spontaneously. Whether the staphylococcal infection played a role in the pathogenesis of our case remains uncertain.

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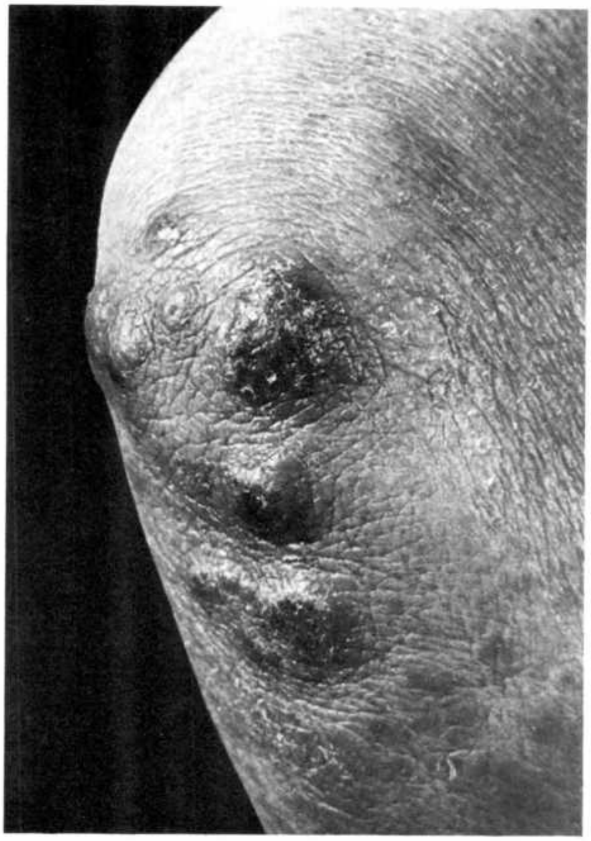

FIGURE 8. Erythematous nodules on right elbow.

Generalized Pagetoid reticulosis or mycosis fungoides?

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History. Male, aged 61 years. In 1975 the patient presented with a lesion on his right upper leg of one year's duration which was diagnosed as mycosis fungoides. Local X-ray and mechlorethamine led to complete remission. In November 1981 he presented again with generalized itching and skin lesions of 6 months' duration.

Examination. Mainly in both axillary regions, and to a lesser extent dispersed over the trunk and extremities, several single and confluent red infiltrated lesions of variable size were observed. Some of the lesions had an annular configuration with a pale centre and a red, raised infiltrated border (Fig. 9). There was no lymphadenopathy or hepatosplenomegaly.

Laboratory tests. No abnormalities were found, particularly no circulating Sézary cells.

Histology. The original biopsy of 1975 showed a classical picture of mycosis fungoides with a broad band-like infiltrate in the upper dermis with many Sézary cells and occasional Pautrier microabscesses. Biopsies taken in November 1981 and during the follow-up showed a strikingly different picture. In the acanthotic epidermis several well-demarcated cavities were seen, containing characteristic Sézary cells, blast cells and lymphoid cells with more or less irregular or nearly round nuclei (Fig. 10). In the stratum

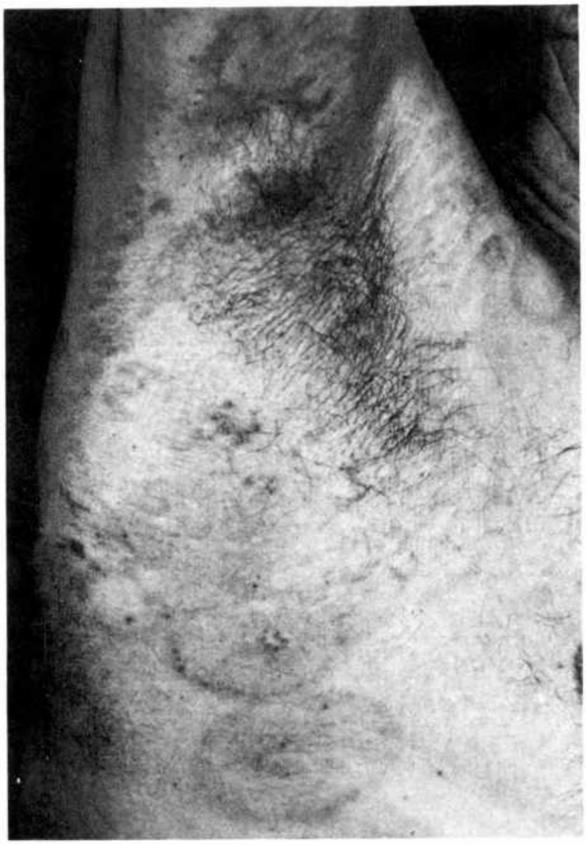

FIGURE 9. Infiltrated, partly annular, lesions in right axillary region.

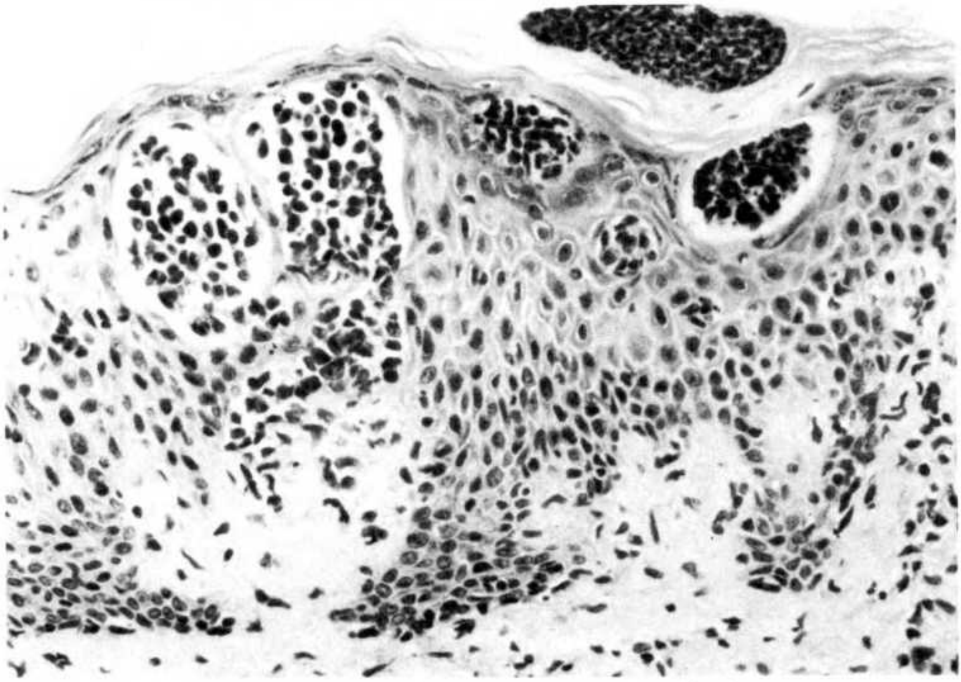

FIGURE 10. Pautrier-like microabscesses in the epidermis.

corneum nests of these cells could be observed. Lymphoid cells with indented nuclei were regularly found between the basal cell layers of the epidermis. A sparse lymphohistiocytic infiltrate with occasional Sézary cells was present in the papillary dermis, and around the dermal vessels. On electron microscopy a few Langerhans cells could be detected in the abscesses, often in close apposition to lymphoid cells.

The immunological phenotype of the lymphoid cells (Leu 1^+, Leu $2a^-$, Leu $3a^+$, Leu 5^-, OKIa$^+$) showed they were activated helper T cells. One or two OKT6-positive Langerhans cells were present in each abscess. Biopsies taken in 1983 and 1984 showed in some sections a classical picture of mycosis fungoides, whereas other sections showed only the pagetoid changes described previously.

Treatment. Treatment with UV-B and local steroid cream (Diprosone®) led to an almost complete remission. After stopping the UV-B the lesions recurred after several weeks.

Discussion. Generalized Pagetoid reticulosis (PR) is a very rare disease. It was first described by Ketron & Goodman (1931). In contrast to the localized form (Woringer–Kolopp) PR occurs at an older age and has a worse prognosis (Braun-Falco *et al.*, 1979). Several authors consider generalized PR to be an epidermotropic variant of mycosis fungoides (Degreef *et al.*, 1976; Haneke, Tulusan & Weidner, 1977). This is supported by the present case who shares the features of classical mycosis fungoides as well as generalized PR.

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Bullae in diabetics

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History. Male, aged 59 years. The patient was known to have had diabetes mellitus since 1968, complicated by retinopathy, polyneuropathy, neurotrophic ulcers on the feet and hypertension. Since 1980 the patient had noticed recurrent bullous lesions restricted to the hands. The bullae developed spontaneously without preceding trauma. Healing occurred without scarring within several weeks. Recently a rectal carcinoma was removed surgically, without influence on the occurrence of new bullae. The diabetes was controlled by insulin, the hypertension by chlorthalidone and methyldopa.

Examination. Both index fingers showed tense bullae with a clear serous content (Fig. 11). The skin of the fingers was tight, thick and waxy.

Laboratory tests. Bacteriological examination of the blisters was usually negative. Occasionally *Staphylococcus aureus* was cultured. Blood sugar levels were elevated (range 10–17 mmol/l). Serum urea and creatinine were slightly elevated. Coproporphyrin and uroporphyrin excretion in a 24-hour urine sample were normal.

Histology. The peribullous skin of the subepidermal blister showed focal spongiosis in the stratum spinosum. No pathological changes were seen in the dermis. Some papillary blood vessel walls showed PAS-positive material. Immunofluorescence of the skin was negative.

Treatment. Secondary infection was treated.

Discussion. Bullae are a rare skin manifestation of diabetes. The lesions are mostly confined to the distal extremities, mainly to the feet. Lesions restricted to the hands have been reported only by Bear & Wall

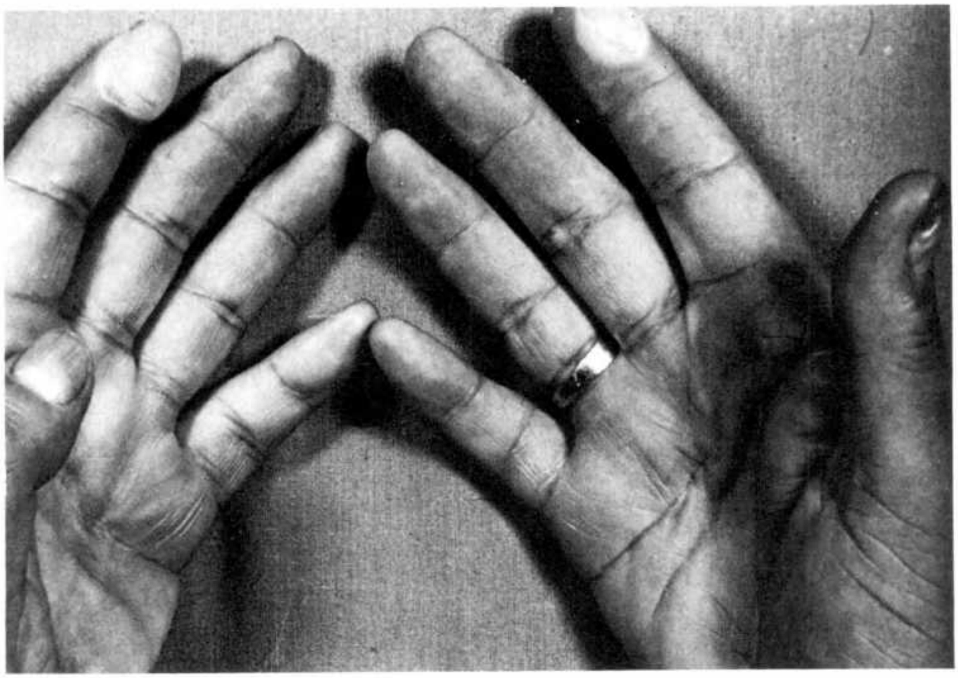

FIGURE 11. Index fingers: tense bullae filled with clear fluid, arising in skin which appears normal.

(1969). The pathogenesis is obscure, but possible factors include vasculopathy, neuropathy, disturbance in the carbohydrate metabolism or a combination of these factors (Cantwell & Martz, 1967). Recently a reduced threshold to suction-induced blister formation was demonstrated in insulin-dependent diabetics (Bernstein *et al.*, 1983).

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Multiple cutaneous immunocytomas and type III cryoglobulinaemia

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History. Male, aged 61 years. Since 1977 the patient has suffered from recurrent skin lesions on the trunk and extremities. Some lesions cause local itching but the majority are asymptomatic. Within months they show spontaneous regression. During the same period the patient has developed slowly progressive renal failure due to glomerulonephritis. A type III cryoglobulinaemia has been found.

Examination. Dispersed over both arms, the trunk and the right upper leg, six plaque-like infiltrates of various sizes (range 6–10 cm) were found. Their colour ranged from pale blue-grey to brownish-red. On palpation they showed a marked induration. A large prominent tumour of about 6 cm was present on the extensor side of the left lower arm (Fig. 12). Neither lymphadenopathy nor hepatosplenomegaly was observed.

Laboratory tests. E.S.R. 138 mm/h, serum creatinine 300 μmol/l. Albuminuria (3–5 g/24 h). Increased γ-globulin, mainly IgM, range 8–35 g/l, IgG 15 g/l, IgA 3–5 g/l. There was no paraproteinaemia. Cryoglobulins were demonstrable at 4°C and to a lesser degree at 20°C, consisting of polyclonal IgG and IgM. Complement values were usually decreased (C1q 0–59%, C3 28–66%, C4 19–70%).

Histology and immunology. Tumour of left arm: a normal flattened epidermis and free subepidermal zone. Dermis: a dense, dermal infiltrate extending into subcutaneous fat consisting of lymphoid cells of normal size with irregular nuclear indentations. The cell morphology resembled that of centrocytes. Mature lymphocytes and plasma cells were intermingled. Few mitotic figures were present. Other biopsies showed similar pictures, but with more plasmacytoid cells. In lymphoid cell suspensions, prepared from biopsies, 68% of the cells bore IgA lambda on the cell membranes. Sections of sublimate-formaldehyde fixed samples: a large majority of the cells was also positive for cytoplasmic IgA lambda. Uninvolved skin: granular deposits of IgM (containing both kappa and lambda light chains), C1q and C3 along the basement membrane and in the walls of dermal blood vessels. Kidney: a mesangio-capillary glomerulonephritis with granular deposits of IgG, IgM, C1q, C3 and C4 along the glomerular basement membrane and in the mesangium. Bone marrow: no cytomorphological abnormalities. However, the majority of plasma cells were IgM positive (ratio IgA:IgG:IgM = 23:21:56; normal distribution 40:50:10) with a shifted kappa/lambda ratio (50:50; normally 65:35).

Treatment. As the tumours showed spontaneous regression, the patient received no treatment.

Discussion. This patient shows a unique combination of isolated multiple cutaneous immunocytes, type IgA lambda and a type III cryoglobulinaemia. This mixed type cryoglobulinaemia apparently caused complement activation and was associated with an immune complex glomerulonephritis. The combination of type III cryoglobulinaemia and malignant lymphoma has been described, but not in combination with

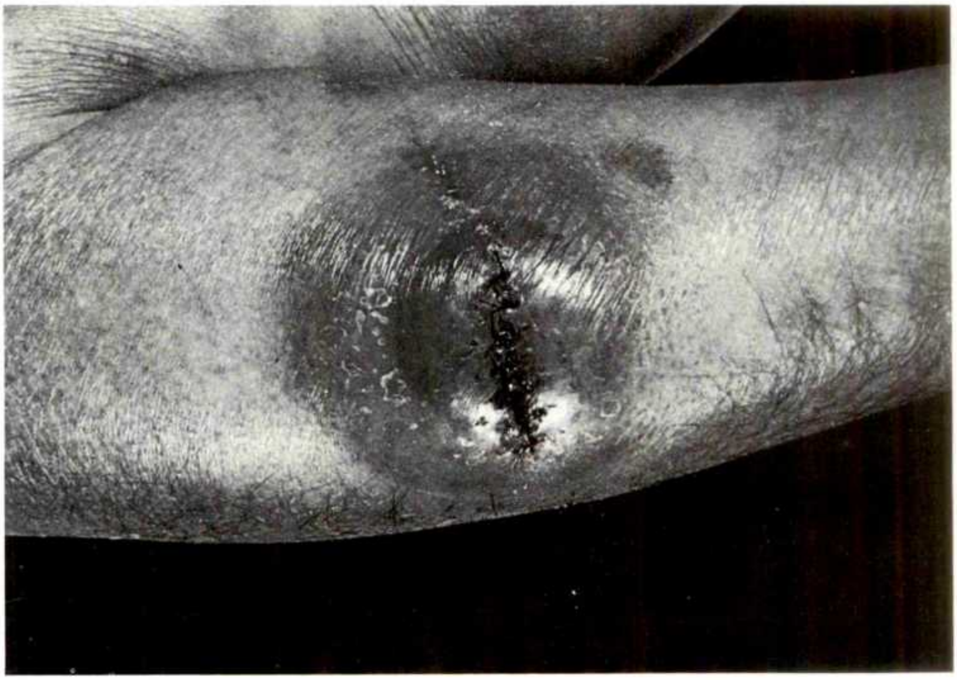

FIGURE 12. Immunocytoma on left forearm (after surgical biopsy).

cutaneous immunocytomas (Brouet *et al.*, 1974). The majority of cutaneous immunocytomas produce IgM kappa (Kerl & Burg, 1979; Guggenberger *et al.*, 1982). The IgA lambda type has not been reported before. Whether a relationship exists between the skin lesions and the cryoglobulinaemia remains uncertain.

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Epidermolysis bullosa herpetiformis Dowling–Meara

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History. Girl, aged 9 months. Since birth blisters and infected erosions have been present on face, hands, feet, around the umbilicus and nipples, buttocks and on the oral mucosa. The nails reacted with transient dystrophy on recurrent blistering. Two teeth showed caries. Pregnancy and delivery were normal.

Family history. The mother (33 years of age) has suffered from a bullous disease since birth. Over the years, the number and size of new lesions decreased. Her palms and soles became hyperkeratotic. The scalp hair was fragile. The nails of the feet were dystrophic. The teeth were normal. The patient's maternal grandfather suffered infrequently from plantar blisters. This condition seemed to have an autosomal dominant inheritance. There was no consanguinity.

Examination. Tense serous bullae were seen, about 0·5 cm diameter, arranged in groups, mainly on pressure areas. Healing occurred with milia and slight atrophic scarring.

Laboratory tests. Leukocytes $25{\cdot}4 \times 10^9$/l; Hb 14·5 mmol/l. All other investigations (blood, urine, faeces) were within normal limits. The erosions were frequently contaminated with staphylococci and yeasts.

Histology. A subepidermal blister was observed, no increase of eosinophils. Immunofluorescence was negative.

Electron microscopy. There was clumping of tonofilaments with suprabasal cleavage due to cytolysis (Anton-Lamprecht & Schnyder, 1982).

Treatment. Silver–sulphadiazine was applied locally and selective bowel decontamination with antibiotics was started to control superinfection. Later only trichloric acid in a polyethylene glycol wax was given. Dietary measures were taken and transfusions were given to replace loss of proteins and electrolytes through blister fluid.

This case will be published in detail elsewhere.

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Epidermolysis bullosa, Herlitz, non-letalis

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History. Male, aged 22 years. At birth he had a bullous eruption on the hands, elbows, feet, knees and buttocks. Later lesions arose after minor trauma. Severe caries necessitated total dental extraction. His intelligence was subnormal.

Family history. His mother (66 years of age) had suffered also from blisters since birth. The overall number decreased during her life. She was edentulous and showed widespread alopecia. His father was healthy. Six children were born: two died, respectively at 2 and 4 months with an almost 'denuded' skin; the other children were unaffected.

Examination. Flaccid bullae with serous fluid and erosions on pressure areas were seen. Healing occurred with atrophic scars. Nikolsky's sign was positive. The nails were all dystrophic. The hair was strawlike. His stature was dysmorphous and marfanoid.

Laboratory tests. Blood-cell count, urinanalysis and biochemical screening were normal. There was a low serum albumin content. The lesions were frequently contaminated with staphylococci, streptococci, proteus and *E. coli*. X-ray of thorax and ECG were normal. CT-scan of brain showed signs of atrophy. Chromosomal analysis: 46 XY, normal pattern.

Histopathology. A subepidermal blister was seen.

Direct immunofluorescence: negative.

Electron microscopy: there was a junctional blister (Anton-Lamprecht & Schnyder, 1979).

Heredity. The parents were related, as is often seen in this autosomal recessive type of epidermolysis bullosa.

Treatment. Trichloric acid in a polyethylene glycol wax was applied locally. Antibiotics were administered according to bacterial sensitivity. Phenytoin, a drug in use for dystrophic epidermolysis bullosa, was recently suggested for the junctional variant of EB (Rogers *et al*., 1983). During this therapy (at a blood concentration of 8 μg/ml) our patient developed a pseudo-lymphomatous reaction (Charlesworth, 1977), but there was no effect on his skin condition.

This case will be published in detail elsewhere.

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Carcinoma telangiectaticum

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History. Female, aged 60 years. In January 1981 the patient noticed a blue-purple discoloration of her forehead. She also complained of headache and loss of weight (25 kg during the last 6 months).

Examination. In the mid-forehead a 10 × 7 cm eruption with a sharp border was present, consisting of erythema, telangiectases, venular dilatation and slight oedema (Fig. 13). The lateral side of the cornea and the epipharynx also showed telangiectases. Firm lymph nodes were palpable; two cervical on the left side and one supraclavicular on the right side. In the lateral upper quadrant of the right breast a 3·5 × 4 cm tumour was present, fixed to the skin.

Laboratory tests. Serum: alkaline phosphatase 149 U/l, LDH 262 U/l. Total body-scan: multiple 'hotspots' characteristic of metastases. Further investigations showed no abnormalities.

Histology. The forehead skin had wide dermal blood vessels with extensive tumor embolism (Fig. 14). Breast tumour: infiltrating ductal carcinoma, oestrogen receptor positive.

Treatment. After local excision of the breast tumour, the patient was treated with ethinyloestradiol 3 × 1 mg/day in May 1981. Remission followed, but in August 1982 bone-scan abnormalities recurred. After withdrawal of therapy a shorter remission followed until February 1983. Subsequently tamoxifen 3 × 20 mg/day was started, resulting in regression of the bone-scan abnormalities. Venectases were still present on the forehead but tumour embolism had disappeared. Progression of bone metastases was again noted in September 1983. The hormonal treatment was changed to aminoglutethimide 4 × 250 mg/day with hydrocortisone 40 mg/day.

A clinical improvement occurred together with a decrease of the elevated blood alkaline phosphatase level. In January 1984 the patient died rather suddenly, probably due to toxic septicaemia, possibly in combination with a pulmonary embolism. Autopsy was not permitted.

Discussion. To our knowledge, carcinoma telangiectaticum localized to the forehead has not been described before.

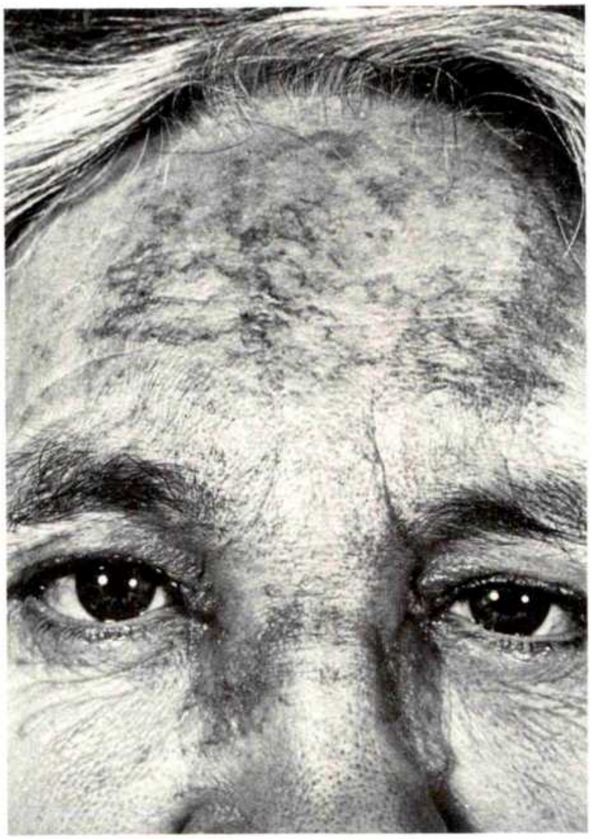

FIGURE 13. Telangiectases, venular dilatation, erythema and slight oedema.

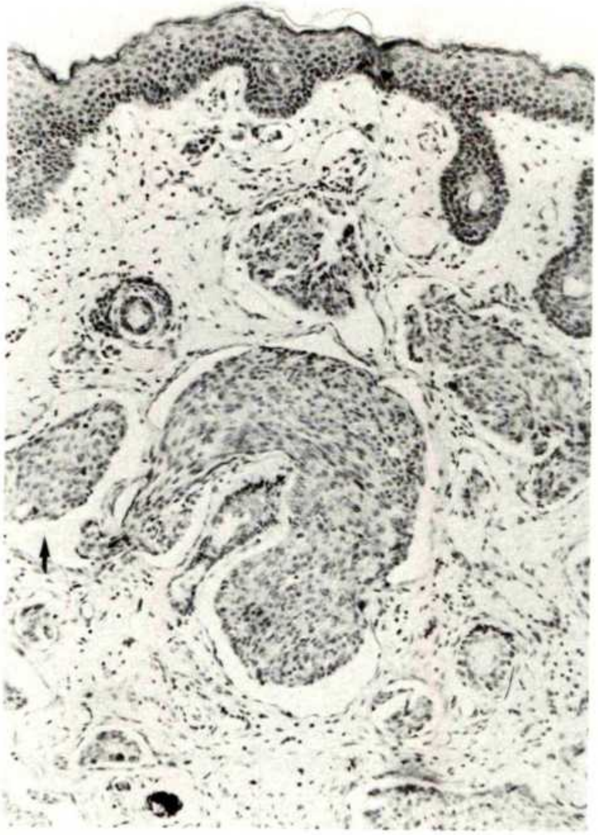

FIGURE 14. Tumour embolism in wide dermal vessels.

British Journal of Dermatology (1984) **111**, 499–502.

Correspondence

Metronidazole suspension applied topically for rosacea

SIR, Oral metronidazole is effective in rosacea (Pye & Burton, 1976; Schirner & Haneke, 1981), and is equivalent in efficiency to tetracyclines (Saihan & Burton, 1980). It has also been demonstrated to be effective topically as a 1% cream (Gamborg Nielsen, 1983a,b). Metronidazole cream is not yet commercially available and requires specific formulation by retail or hospital pharmacy, but a 5% suspension for oral use is available. Centrifugal massage is also stated to be helpful in rosacea (Marks, 1976; Wilkinson, 1968).

An open trial has been conducted in rosacea using metronidazole 5% suspension topically. The patients were instructed to rub the medication in centrifugally 2 or 3 hours before bedtime and wash off before going to bed. Eleven females and four males have been treated for up to 3 months. There has been a marked reduction in papules and some diminution in the erythema in nine females and two males. Some patients had objected to the sugary aspect of the suspension but this is obviated by using it at night and washing it off before going to bed.

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Superficial X-ray therapy in the treatment of palmoplantar pustulosis

SIR, Patients with chronic disabling palmoplantar pustulosis are frequently treated with radiotherapy, although the efficacy of this therapy has not been assessed in placebo-controlled trials. We now report such a study.

Nine symmetrical, paired sites of persistent palmoplantar pustulosis were studied in three patients with involvement of the palms and soles, two patients with involvement of the hands and one patient with involvement of the soles. In a double-blind fashion, according to a predetermined random code, one site was given a dose of 100 rad (1 Gy) of conventional superficial X-ray therapy at 50 kV and the other site received a placebo dose. Each dose was administered three times at intervals of 21 days, using a Siemens Dermopan 2 machine with a 1 mm aluminium filter and a focal skin distance of 30 cm. Any topical therapy was continued for both sites. The patients were reviewed 6, 9 and 18 weeks after the start of X-ray therapy. During each visit the patient and observer separately graded the severity of the disease on both sites (Fairris *et al.*, 1984) and the results were analysed using the paired Student's *t*-test. The patient also recorded which site had improved the most since the start of X-ray therapy and these results were analysed by McNemar's test.

Only one patient with palmoplantar pustulosis on the hands remitted during the 6 weeks of X-ray therapy. The remainder showed no improvement on treated or untreated sites (Table 1). The mean grade of the palmoplantar pustulosis was 8·1 (s.d. 1·7) on both sites at the start of the trial and was 8·6 (s.d. 1·7) and 8·7 (s.d. 2·8) on the sites receiving active and placebo X-ray therapy 9 weeks after the start of therapy. A previous study of hand eczema (Fairris *et al.*, 1984) had shown that this would be the time when maximum improvement after superficial X-ray therapy would be expected.

TABLE 1. Results of the X-ray therapy—patient preference

	Weeks		
	6	9	18
X-ray better than placebo	1	0	1
Placebo better than X-ray	0	0	0
No improvement with either therapy	8	9	8

This trial indicates that conventional superficial X-ray therapy has little or no therapeutic effect upon palmoplantar pustulosis. The hazards of conventional superficial X-ray therapy are well known (Rowell, 1973) and its risks appear to outweigh the meagre benefit in the treatment of persistent plamoplantar pustulosis.

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ROWELL, N.R. (1973) A follow-up study of superficial radiotherapy for benign dermatoses: recommendations for the use of X-rays in dermatology. *British Journal of Dermatology*, **88**, 583–590.

Superficial X-ray therapy in the treatment of constitutional eczema of the feet

SIR, In a recent paper (Fairris, Mack & Rowell, 1984) we reported the results of a placebo-controlled trial of conventional superficial X-ray therapy in the treatment of chronic symmetrical constitutional eczema of the hand. We now report our findings in the treatment of chronic symmetrical eczema of the feet.

Fourteen patients were studied. Cases of contact allergic dermatitis were excluded. In a double-blind, randomized manner, one foot received 100 rad (1 Gy) at 50 kV of conventional superficial X-ray therapy whilst the other foot received a placebo dose. Previous topical therapy was continued to both feet. The X-ray therapy was administered three times at intervals of 21 days using a Siemens Dermopan 2 machine with a 1 mm aluminium filter and a focal skin distance of 30 cm. The patients were reviewed 6, 9 and 18 weeks after the first exposure to X-ray therapy and the severity of the eczema of each foot was assessed independently by the observer and the patient using published criteria (Fairris *et al.*, 1984). The results of both grading systems were analysed using the paired Student's t-test. The patient also recorded which foot had improved the most since the first exposure to X-rays and these observations were analysed by McNemar's test.

There was a significant decrease in the mean grade of the eczema on both active and placebo-treated feet during the 6 weeks of therapy and this was maintained during the 12 week follow-up period (Figs 1 and 2). This improvement was shown by both the grading system used by the observer ($P < 0.001$) and the grading system used by the patient ($P < 0.001$) 18 weeks after the treatment. However, although the actively treated side improved more than the placebo-treated side (Figs 1 and 2), this trend did not reach statistically significant levels. Although five of the fourteen patients preferred the therapeutic result produced by active X-ray treatment (Table 1), this was not a statistically significant finding.

We have previously shown that superficial X-ray therapy improves constitutional eczema of the hand

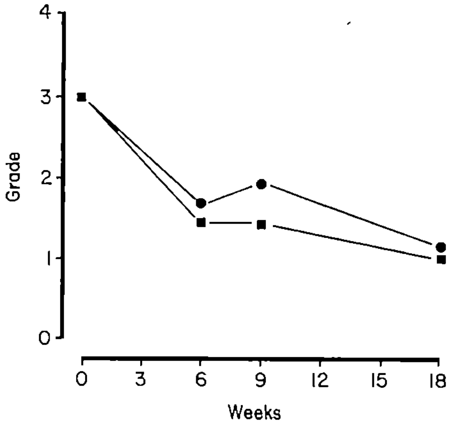

FIGURE 1. Observer's mean grade of eczema on foot receiving active X-ray therapy (■) and on foot receiving placebo (●).

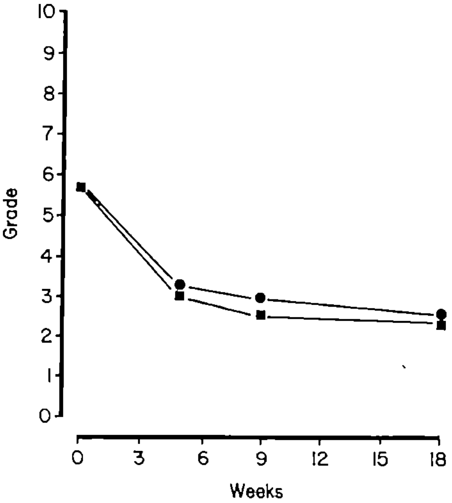

FIGURE 2. Patients' mean grade of eczema on foot receiving active X-ray therapy (■) and on foot receiving placebo (●).

TABLE 1. Results of X-ray therapy—patients' preference

	Weeks				
	6		9		18
X-ray better than placebo	5	NS	5	NS	2
Placebo better than X-ray	1		0		0
Equal response	8		9		12

NS = not significant.

with maximum benefit 6 to 9 weeks after the first treatment (Fairris *et al.*, 1984). The effect was still detectable 12 weeks after the last dose. From the present study radiotherapy appears to have a more limited effect upon constitutional eczema of the feet. This relative lack of effect of radiotherapy upon the foot compared with the hand cannot be due to inadequate penetration, as superficial radiotherapy at 50 kV has a half layer value in tissue of 13 mm.

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D.H.JONES
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N.R.ROWELL

REFERENCES

FAIRRIS, G.M., MACK, D.P. & ROWELL, N.R. (1984) Superficial X-ray therapy in the treatment of constitutional eczema of the hands. *British Journal of Dermatology*, **111**, 445–449.

British Journal of Dermatology (1984) **111**, 503–504.

Book Reviews

Lecture Notes on the Infectious Diseases. By B.K.MANDAL AND R.MAYON-WHITE (1983) 4th edition. Oxford: Blackwell Scientific Publications. Pp. 288. Price £6.50.

This excellent little book has now reached its fourth edition and its breadth has been extended even further by the two authors. The book is very well written, succinct and immensely practical and the essential features of diagnosis and management of communicable diseases are appropriately delineated.

The authors have wisely reduced the chapter size on smallpox and vaccinia, but not omitted reference to this one-time scourge whose eradication represents one of the main triumphs of Western Medicine.

The introduction of a vaccine against hepatitis B and the appearance of acyclovir for the treatment of herpetic infections certainly have far-reaching implications in the prevention and treatment of virus infections and the authors rightly stress this. Our knowledge on human Campylobacter infections has expanded enough to warrant a new chapter, and chlamydial and rickettsial infections deservedly have sections of their own. It is surprising that a book of this breadth, that includes a short account of fungal infections of the skin, does not make mention of cryptococcosis, sporotrichosis and perhaps other not uncommon fungal infections.

There is little doubt that this book will be very useful to students and medical practitioners in many parts of the world.

T.ROBINSON

Handbook of Dermatologic Treatment. By R.K.LANDOW (1983) Greenbrae, California: Jones Medical Publications. Pp. 219.

There is a multitude of handbooks and pocketbooks on dermatology but few are devoted primarily to treatment. Unlike some, this little book does just fit into a pocket and is therefore a convenient travelling companion and vade-mecum.

Every country has its own preferences for treatment and it is inevitable that there should be differences of emphasis between practice in the United States and Great Britain. But there is nothing here that one can take exception to, and a great deal to commend. Dr Landow is a very experienced physician and his approach to therapy is well balanced and laced throughout with common sense. To quibble over details or minor omissions would be unfair when so much information is presented in so small a space.

Dr Landow is too modest to suggest that this book might also be useful for the clinical dermatologist in everyday practice, but it certainly serves as a ready source of reference to the various alternative forms of therapy available. I recommend it.

D.S.WILKINSON

Topics in Photomedicine. Edited by K.C.SMITH (1984) New York: Plenum Press. Pp. 403. Price U.S. $65.

At least three chapters in this book hold plenty of interest for the dermatologist. First, there is Fred Urbach on UVR and skin cancer, nearly 90 text pages and over 250 references; this sounds daunting, but is written with a disarming zest. The five-page introduction sets the scene well and later sections then deal at length with the underlying mechanisms of skin erythema and non-melanoma skin cancer including the role of immune processes. Several interesting tables and graphs relate skin cancer prevalence to solar UV-B exposure. Lastly there is an extended section (some 25 pages) on the many enigmas of malignant melanoma. Recommended reading.

Second are Giulio Jori and John Spikes on porphyrins and photosensitization. Written in an easy English style, it is a sound but painless introduction to a complex field. For the clinician there is a section on tumour photodiagnosis and phototherapy with porphyrin; for the physical chemist, lists of photochemical data. Cognoscenti will find much of interest in the 100 pages of text with its numerous tables, diagrams, figures, and well over 500 references! The novitiate should not fear starting here before proceeding to such 'heavies' as David Dolphin's or Kevin Smith's compilations. A valuable review.

The third contribution of clinical interest is by Kiki Hellman and colleagues, who review immunological defects in human radiation-sensitive disorders, namely in ataxia telangiectasis, xeroderma pigmentosum, Fanconi's anaemia, Cockayne's and Bloom's syndromes and SLE. The review also covers aspects of neoplasia and mutagenesis.

Probably of more direct interest to non-dermatological photobiologists are the two remaining chapters by Coohill and Rodiguiero respectively. Thomas Coohill in some 30 pages has reviewed material on *in vitro* action spectra of mammalian cells from almost 100 references in the literature. This includes data for normal human and xeroderma pigmentosum cells, also various mammalian cell lines for neoplastic and anchorage transformation, mutation and various modes of viral transformation. Nearly all results point to a DNA chromophore. For action spectra longer than 300 nm the literature is thin and partly contradictory.

The last chapter is on monofunctional furocoumarin derivatives. The compilations by Rodighiero and his colleagues of physico-chemical data, such as psoralen–DNA binding constants, etc. are an especially useful feature. It is churlish to complain that the literature references to the more biological aspects, viz. mutation and carcinogenesis tend to the old fashioned and mostly up to 1981, for this region is at present in a turmoil of activity. Unluckily also, Coohill's review above just pre-dates the work of Giannelli's group at Guy's on human UV-A sensitive cells in 'actinic reticuloid' type dermatoses.

Verdict: this is a generally high-standard production, which should allay the fears of those who feel that too much photobiology verbiage is being churned out at the moment. The editorial ploy of 'in-depth coverage of selected topics' (to quote the Preface) seems wiser than attempting complete blanket treatment as in some very recent treatises. The cost in the UK seems unknown ($65 in USA), but £50 or so for just the first 3 chapters reviewed above seems reasonable.

I.A.MAGNUS

Diagnostic and Basic Aspects of Cutaneous Lymphomas. REIN WILLEMZE (1983) Thesis. University of Leiden, The Netherlands, Pp. 144.

The Continental habit of presenting a thesis in the form of a number of already published papers with a common theme, and linked together by an introductory and final chapter respectively, has much to recommend it. In many British theses only small subsections of the work are ever published, but in contrast, in the Continental ones a much greater bulk is more widely available for inspection.

Dr Willemze's thesis illustrates the value of this approach very well indeed. His theme is the use of modern techniques to further refine our diagnostic ability in cutaneous lymphomas. Of the six middle chapters which are the meat of the thesis, three have already been published in the *British Journal of Dermatology*, one in *Cancer*, one in the *Journal of Investigative Dermatology*, and one is currently in press in the *J.I.D.* All chapters are of a high quality and illustrate well this Dutch group's interest and expertise in the area of cutaneous B and T cell lymphomas, the Sézary syndrome and lymphomatoid papulosis.

RONA M. MACKIE

Thesis received

Optical Properties of Human Epidermis, by W.A.G. Bruls, Anth. Matheuslaan 13, 3515 AN Utrecht, Holland.

British Journal of Dermatology (1984) **111**, 505–506.

News and Notices

Alfred-Marchionini Prize, 1987

The Alfred-Marchionini Prize (value DM 20,000) will be awarded for the second time at the XVII Congress of Dermatology in West Berlin in September 1987. The prize will be awarded to a young scientist in the field of medicine, biochemistry, or biopharmacy, and it will be awarded after taking into account the candidate's contribution to international human relations as well as his or her scientific work. The proposals for this prize must include a detailed appreciation of the candidate with supporting documents. Submissions are invited from medical directors of hospitals, research or other scientific institutes, and they should be addressed to the Secretariate of the Alfred-Marchionini-Stiftung, Danziger Str. 5, D-2056 Reinbek, Germany.

Therapeutic Photomedicine, Vienna, February 1985

This clinically oriented symposium, sponsored by the ESDR and the Vienna Academy of Postgraduate Medical Education, will take place in Vienna, Austria on 10–12 February 1985. The organisers are Herbert Honigsmann, M.D. and Georg Stingl, M.D.

The main subject areas to be covered are basics of terminology, therapeutic principles, molecular aspects of phototherapy, the role of phototherapy in clinical dermatology, immunological aspects of phototherapy and long-term hazards of phototherapy.

There will be several guest speakers, but in addition there will be some selected papers and poster communications. Further details may be obtained from the Congress Office, Vienna Academy of Postgraduate Medical Education, Alser Strasse 4, A-1090 Vienna, Austria.

Poster Winners—European Society for Dermatological Research, Amsterdam, 1984

At the Annual Meeting of the ESDR two poster awards are made. This year the winners of the Bill Reed poster award were H.Torma and A.Vahlquist of the Department of Dermatology, University of Uppsala, Sweden, and the winners of the Ferdinando Serri Foundation Award were J.Schweizer, M.Kinjo, G.Furstenberger and H.Winter, Institute of Experimental Pathology, German Cancer Research Centre, 69 Heidelberg, F.R.G.

Third Annual Consultants' and Senior Registrars' Course, Institute of Dermatology, London, March 1985

A course entitled 'Clinical Dermatology: Recent Advances' will be held on 14–16 March 1985 at the Cumberland Hotel, London W1. Apart from lectures, emphasis will be placed on informal lunchtime discussion groups. There will be an optional evening session in which course members will be encouraged to present brief illustrated accounts of diagnostic or therapeutic problems they have recently experienced for discussion by a panel. For further details please contact the course organiser: Professor M.W.Greaves, Institute of Dermatology, Homerton Grove, London E9 6BX. Tel. 01 985 7061. Early application is advised.

European Society for Dermatological Research. Annual General Meeting and Annual Scientific Meeting, May 1985

The next meeting of the E.S.D.R. will take place at the R.A.I. Congrescentrum, Amsterdam, 12–15 May 1985. The central theme is receptors and the skin. There will be four guest lectures. Papers on other subjects, however, will be welcomed. Abstract forms are obtainable from the Secretary, Dr W.J.Cunliffe, Department of Dermatology, The General Infirmary, Leeds LS1 3EX, U.K. Abstracts must be submitted by 15 January 1985.

Course on Porphyrins and Porphyrias, Barcelona, February 1985

A course on porphyrins and porphyrias will be held in Barcelona in February 1985. The lectures, in English, will be given by an international team of experts. For registration, write to Dr. M.Lecha, Department of Dermatology, Hospital Clinico y Provincial, Casanova 143, 08036 Barcelona, Spain.

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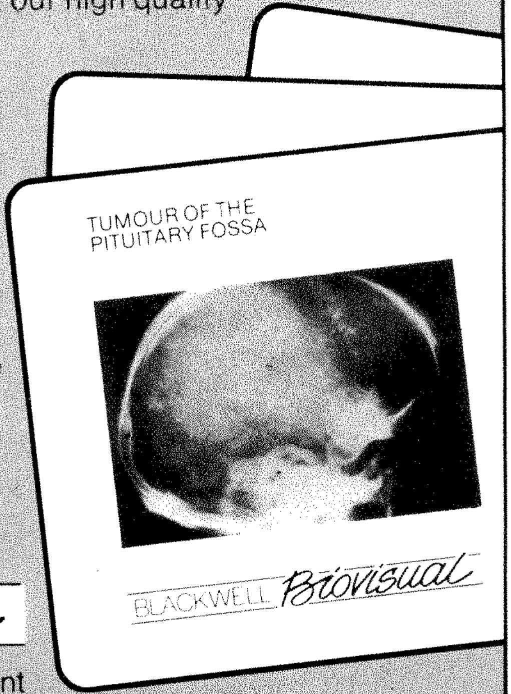

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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This Journal is covered by *Current Contents*, *ISI/BIOMED*, *Science Citation Index* and *ASCA*.

Manuscripts should be sent to the Editor, Dr J.L.Burton, Dermatology Department, Bristol Royal Infirmary, Bristol BS2 8HW. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. A duplicate copy, complete with figures, tables and references, should be sent, since in many cases two referees are consulted. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3}g)	mg	second(s)	s	gravitational acceleration	***g***
microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hours(s)	h	milliequivalent	mmol		

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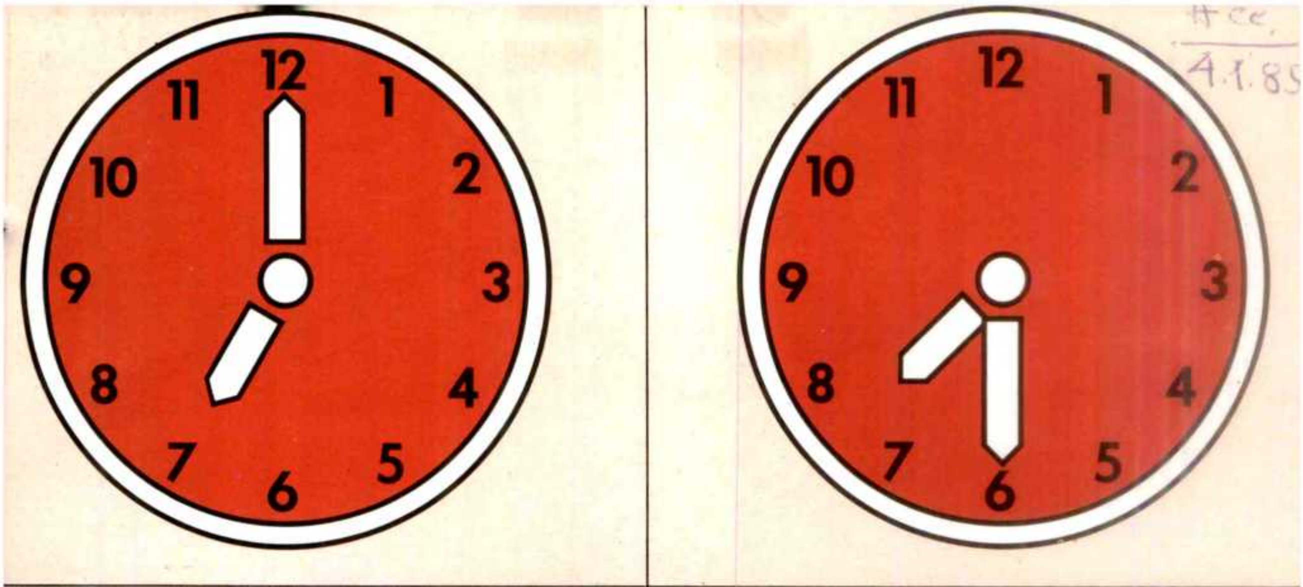

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Dithrocream HP: 1.0% Dithranol BP

INDICATIONS
For the topical treatment of sub-acute and chronic psoriasis including psoriasis of the scalp. Dithrocream Forte (0.5%) and Dithrocream HP (1.0%) should only be used once daily and for those patients who have failed to respond to lower strengths of dithranol. Dithrocream HP should normally only be used for short contact periods.

DIRECTIONS FOR USE
Dithrocream may be applied at night before retiring and washed off in the morning. Alternatively, it may be applied at any convenient time and then removed by washing off, usually no more than one hour after application. Continue treatment until the skin is smooth and clear.

CONTRA-INDICATIONS
Acute psoriasis, dithranol sensitivity.

PRECAUTIONS
Keep away from the eyes. Apply to psoriatic areas only. Wash the hands after use.

PACKAGE QUANTITY
All strengths of Dithrocream are supplied in collapsible tubes containing 50g.

PRODUCT LICENCE NOS
Dithrocream 0.1% : 0173/0029.
Dithrocream 0.25% : 0173/0028.
Dithrocream Forte (0.5%): 0173/0027.
Dithrocream HP (1.0%): 0173/0039.

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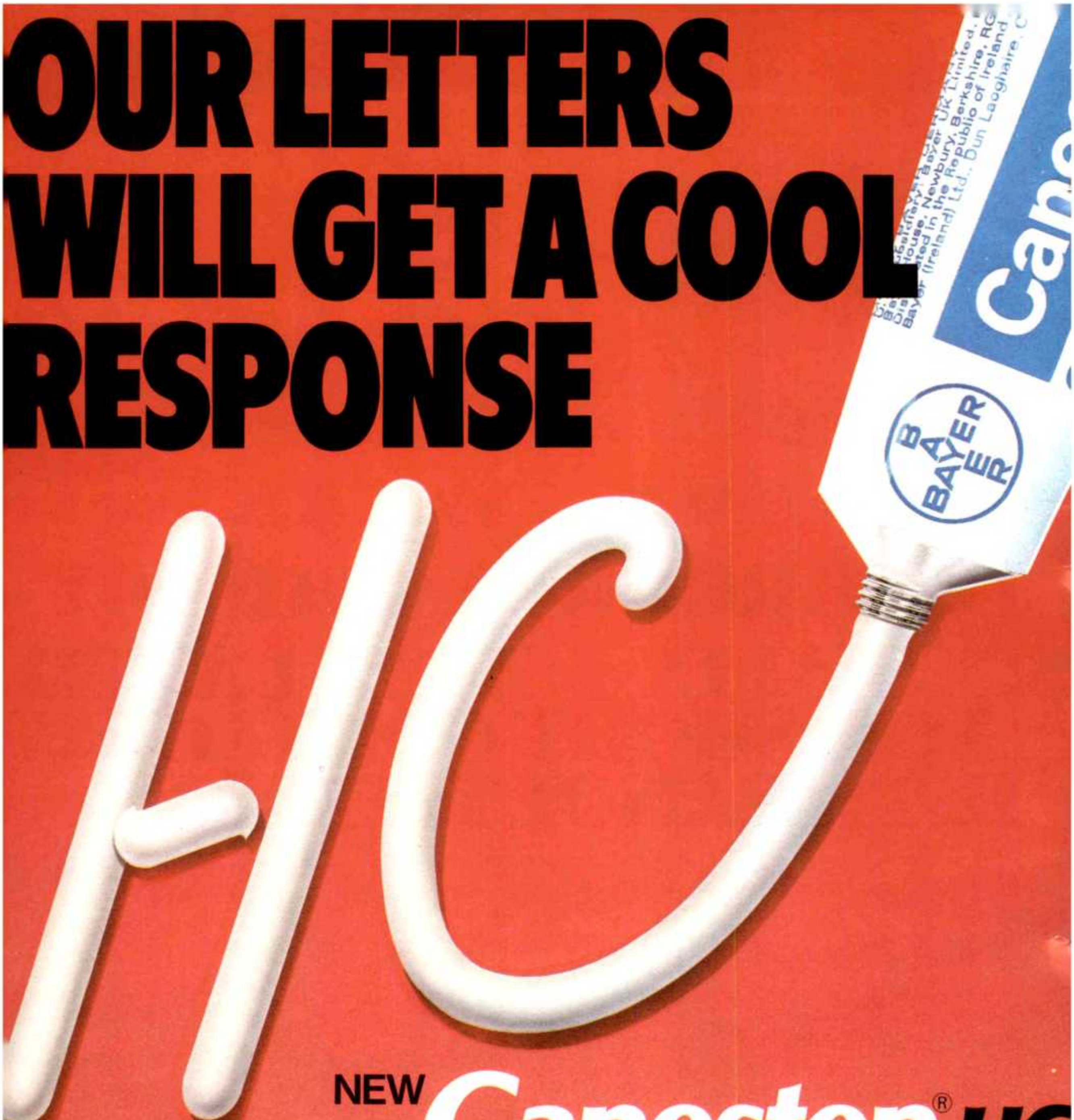

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Canesten and 1% hydrocortisone for itchy, inflamed fungal infections

Prescribing Information – Presentation: A white cream containing 1% clotrimazole and 1% hydrocortisone. **Indications:** All fungal skin infections due to dermatophytes, yeasts, or moulds where there is co-existing inflammation requiring rapid relief. **Dosage and Administration:** Canesten HC should be applied twice daily to the affected areas, and rubbed in gently. **Contraindications:** Hypersensitivity to any of the ingredients. **Side Effects:** Rarely, patients may experience mild local irritation or burning after application. **Precautions:** Care, as with any topical corticosteroid, is advised with infants and children where the preparation is to be applied extensive surface areas or under occlusive dressing. However, continuous prolonged treatment should n be necessary. Topical steroids should not be used extensively in pregnancy. **Basic NHS Cost:** Cream 30g £3.18. **Product Licence Number:** 0010/0120

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Further information is available from:
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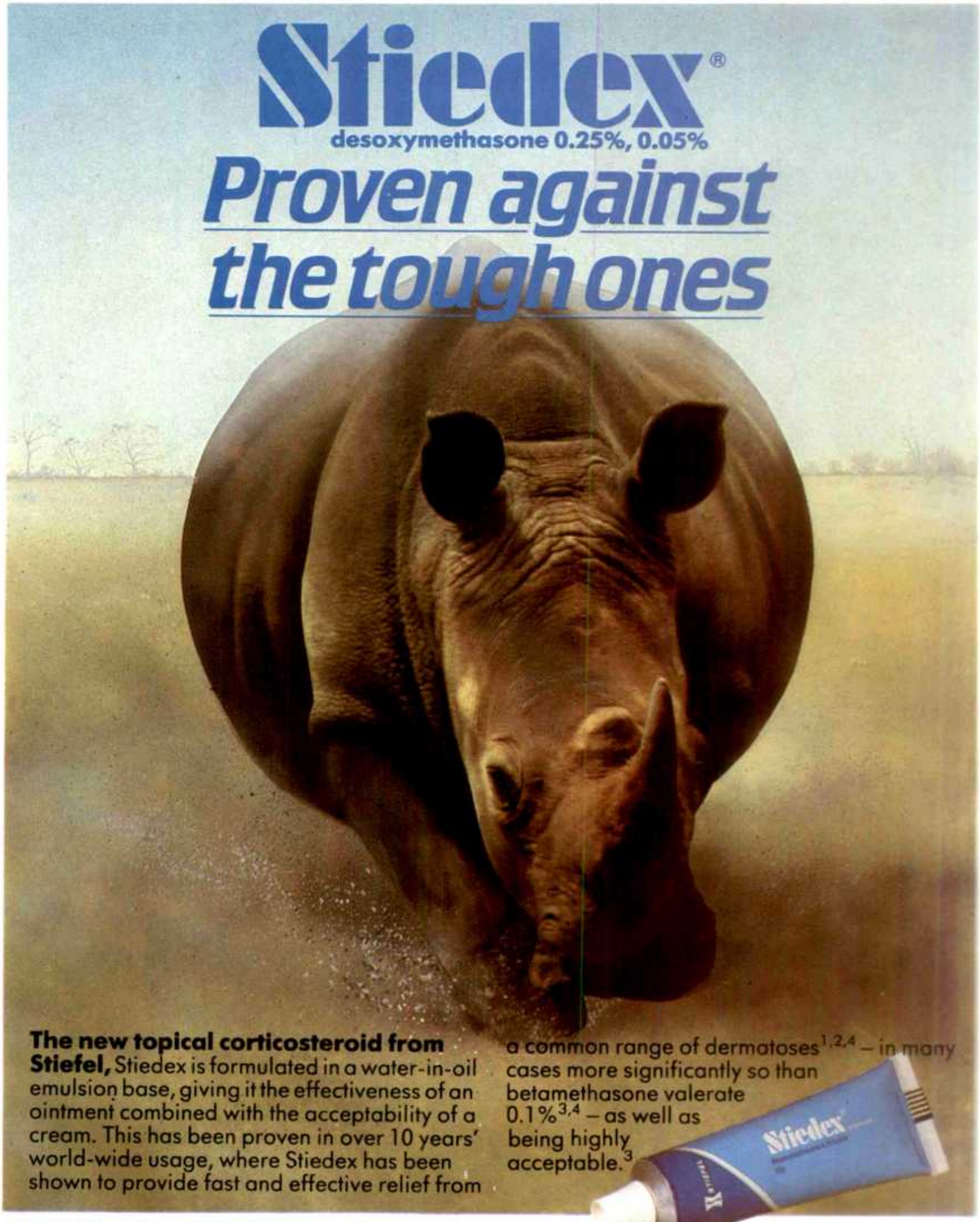

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Full information and data sheet on request
Stiefel Laboratories (UK) Limited, Wellcroft Road, Slough SL1 4AQ.

Prescribing information Stiedex: 0.25% desoxymethasone in an oily cream base; Stiedex LP: 0.05% desoxymethasone in an oily cream base: Stiedex L P N: 0.05% desoxymethasone and 0.5% neomycin (as the sulphate) in an oily cream base. **Uses:** Stiedex: treatment of severe acute inflammatory and allergic conditions; and for chronic skin disorders such as psoriasis. Stiedex L P: treatment of eczema; non-specific treatment of sunburn; insect bites, anogenital pruritis. Stiedex L P N: steroid responsive dermatoses complicated by bacterial infection. **Administration:** apply sparingly to the affected area and rub gently into the skin 2 to 3 times daily. **Contra-indications:** not suitable for infants and young children. Not to be used in primary infective diseases of the skin, in particular, tuberculous, fungal and viral infections, e.g. herpes simplex and zoster, viral warts. Do not use extensively in pregnancy, i.e., in large amounts or for long periods. Not suitable for inflammatory disorders of the eye. Stiedex L P N: not to be used in patients with a known hypersensitivity to neomycin. **Warnings:** Long-term continuous topical steroid therapy should be avoided where possible, particularly in infants and children as adrenal suppression can occur, even without occlusion. Moreover, prolonged administration of corticosteroids causes a reduction in skin collagen and subcutaneous atrophy, resulting in striae, thinning and dilation of superficial blood vessels, especially when the corticosteroid is used on the face. Stiedex, Stiedex L P oily creams may be diluted if necessary, by admixture with Aqueous Cream B.P. **Legal category** POM. **Packs and NHS prices:** Stiedex, tubes of 30g: £3.43, Stiedex L P, tubes of 30g: £2.86, Stiedex L P N, tubes of 15g: £2.17. **PL no's** Stiedex – 0174/0052, Stiedex L P 0174/0053; Stiedex L P N 0174/0054.

References 1. Cutis (1978) **21,** 403 2. Curr. Med. Res. Opin (1977), **4,** 650 3. Curr. Ther. Res. (1978) **24,** 37 4. Drugs (1978) **16,** 302

Monphytol

boric acid, chlorbutol,
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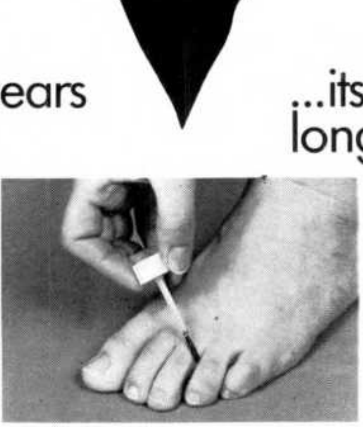

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MONPHYTOL

Presentation: A colourless, rapidly drying, non-greasy paint containing boric acid 2%, chlorbutol 3%, methyl undecylenate 5%, propyl undecylenate 1%, salicylic acid (free and methyl ester) 31%.
Uses: Monphytol is indicated for chronic paronychia, intertrigo, erosio interdigitalis, tinea pedis, tinea circinata, tinea unguium.
Dosage and administration: Four times a day, moisten brush with Monphytol and apply to the affected parts, reaching gently into the folds of the skin. Treatment should be repeated from time to time after the condition has subsided to prevent reinfection.
Contra-indications, warnings, etc: Monphytol may sting sensitive weeping areas of acutely inflamed skin. Other treatment (to reduce inflammation and exudation) may first be necessary. Although there is only a small amount of boric acid present, caution is advised in the repeated application of Monphytol to extensive areas of the skin.
Pharmaceutical precautions: Store away from heat.
Legal category: P
Package quantities: Bottles containing 18 ml with brush fitted in cap.
Further information: Nil.
Product licence number: 0113/5010.
Basic NHS price: £0.57 per 13 ml bottle.

Reference

1. Krebs HA, Wiggins D, Stubbs M, Sols A, Bedoya F. Studies on the mechanism of the anti-fungal action of benzoate. Biochem J. 1983; **214**: 657-63.

Monphytol is a trademark

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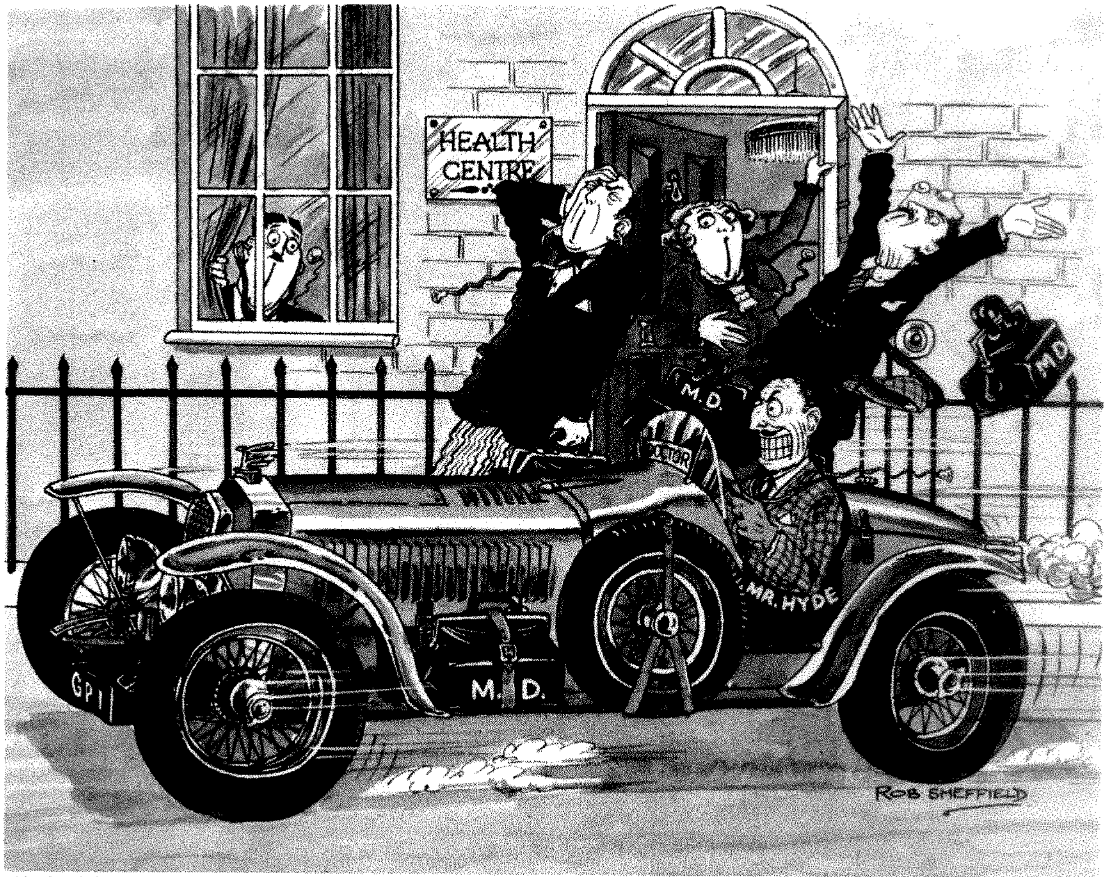

With acknowledgements to H.M. Bateman.

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(betamethasone valerate)

Simply Betnovate, Ready Diluted

Prescribing information

Presentation

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Uses

Inflammatory dermatoses responsive to topical corticosteroid therapy. Betnovate RD preparations are indicated for maintenance treatment when control has been achieved with Betnovate (0·1%) Cream or Ointment.

Dosage and administration

A small quantity of Betnovate RD should be applied to the affected area two or three times daily. Betnovate RD Cream is especially appropriate for moist or weeping surfaces and Betnovate RD Ointment for dry, lichenified or scaly lesions, but this is not invariably so.

Precautions

Long-term continuous topical therapy should be avoided where possible, particularly in infants and children, as adrenal suppression can occur even without occlusion. Appropriate antimicrobial therapy should be used whenever treating inflammatory lesions which have become infected. Any spread of infection requires withdrawal of topical corticosteroid therapy and systemic administration of antimicrobial agents. Topical steroids should not be used extensively in pregnancy, i.e., in large amounts or for prolonged periods.

Side effects

Prolonged and intensive treatment with highly active corticosteroid preparations may cause local atrophic changes in the skin such as striae, thinning and dilatation of the superficial blood vessels. As with other topical corticosteroids, prolonged use of large amounts, or treatment of extensive areas, can result in sufficient systemic absorption to produce the features of hypercorticism. This effect is more likely to occur in infants and children. Betnovate RD preparations are usually well tolerated, but if signs of hypersensitivity appear, application should stop immediately.

Contra-indications

Rosacea, acne and peri-oral dermatitis. Skin lesions caused by infection with viruses (e.g., herpes simplex, chickenpox), fungi (e.g., candidiosis, tinea) or bacteria (e.g., impetigo). Hypersensitivity to the preparation.

Product licence numbers

Betnovate RD Cream: 0004/0277
Betnovate RD Ointment: 0004/0278

Basic NHS cost (exclusive of VAT)
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ESTABLISHED: July, 1979 **PUBLISHED:** Monthly **EDITOR:** J. Graham Smith, Jr., M.D.

The **JOURNAL OF THE AMERICAN ACADEMY OF DERMATOLOGY** is a professional journal devoted exclusively to the clinical and continuing education needs of the entire dermatologic community, including those family practitioners, pediatricians, and internists who are concerned with clinical manifestations of skin disease in their practice.

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Diseases of the Nails and their Management

Edited by **R. Baran** MD, *Head of Dermatological Unit, General Hospital, Cannes,* and **R.P.R. Dawber** MA, MB, ChB, FRCP, *Consultant Dermatologist, Oxford Hospitals and Clinical Lecturer in Dermatology, University of Oxford*

All clinicians involved in the diagnosis and management of diseases of the nails will find this book an essential reference. It presents a comprehensive account of the structure and function of the nails and of the clinical and therapeutic aspects of nail diseases, including surgical treatment where appropriate. Chapters have been contributed by a team of authors of international renown, and the book is lavishly illustrated with colour plates to aid in diagnosis.

Contents: Structure, embryology, comparative anatomy and physiology of the nail *R. P. R. Dawber & R. Baran;* Physical signs *R. Baran & R. P. R. Dawber;* Investigations and some physicochemical properties of nails *R. P. R. Dawber & R. Baran;* The nail in childhood and old age *R. Baran & R. P. R. Dawber;* Fungal (onychomycosis) and other infections of the nail apparatus *R. Hay & R. Baran;* The nail in dermatological diseases *R. Baran & R. P. R. Dawber;* The nail in systemic diseases and drug-induced changes *D. Fenton & J. D. Wilkinson;* Occupational abnormalities and contact dermatitis *R. Rycroft & R. Baran;* Cosmetics: the care and adornment of the nail *E. Brauer;* Hereditary and congenital nail disorders *L. Juhlin & R. Baran;* Nail surgery and traumatic abnormalities *H. Bureau, R. Baran & E. Haneke;* Tumours of the nail apparatus and adjacent tissues *E. Haneke, R. Baran & H. Bureau.*

1984. 544 pages, 318 illustrations (200 in colour). £48.00

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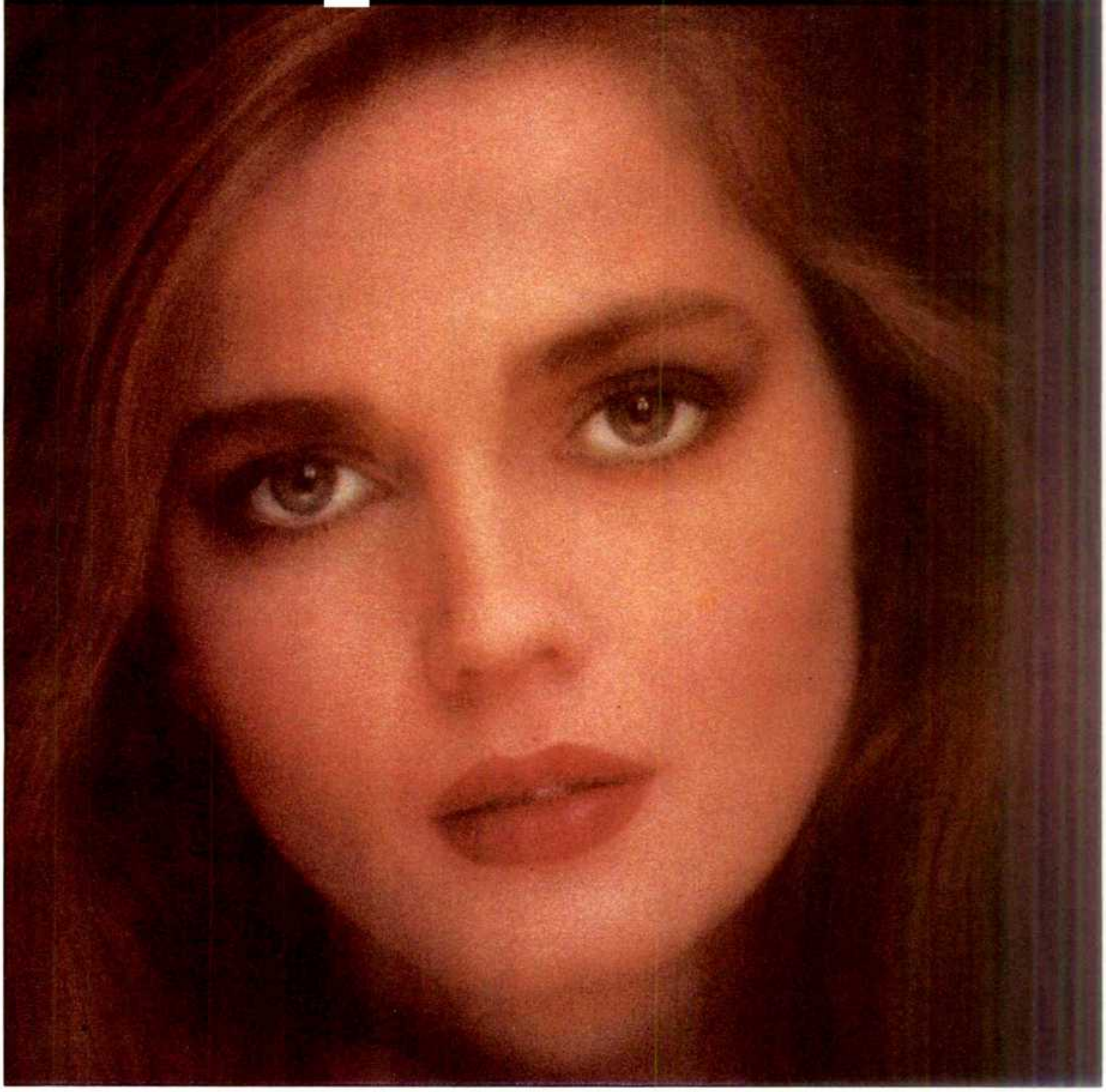

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Arthur Rook MA, MD, FRCP *Consultant Dermatologist, Addenbrookes Hospital, Cambridge*
and **Rodney Dawber** MA, MB, ChB, FRCP *Consultant Dermatologist, John Radcliffe Hospital, Oxford*

This important title, the first work on the subject for a number of years, will be an essential reference for all clinicians involved in the diagnosis and management of diseases concerned with the hair and scalp. It presents a comprehensive account of the hair and its disorders, emphasising their pathogenesis and diagnosis, based on a review of the international literature and the authors' extensive experience.

The first two chapters give a clear account of the embryology, structure and comparative physiology of the hair and the remaining chapters are predominantly concerned with clinical aspects of hair abnormalities.

1982. 567 pages, 197 illustrations. £40.00

Contents: The comparative physiology, embryology and physiology of the human hair; Hair follicle structure and keratinisation and the physical properties of hair; The hair in infancy and childhood; Hair patterns: baldness and hirsutism; Diffuse alopecia: endocrine metabolic and chemical influences on the follicular cycle; Hereditary and congenital alopecia and hypotrichosis; Defects of the hair shaft; Hypertrichosis; Traumatic alopecia; Alopecia areata; Cicatricial alopecia; Hair colour; Infections and infestations of the hair and scalp; Psychological factors and disorders of the hair; Hair cosmetics; Hair and scalp in systemic diseases; Diseases of the scalp and skin diseases involving the scalp; Naevi, tumours and cysts of the scalp; Investigation of hair and hair follicle diseases.

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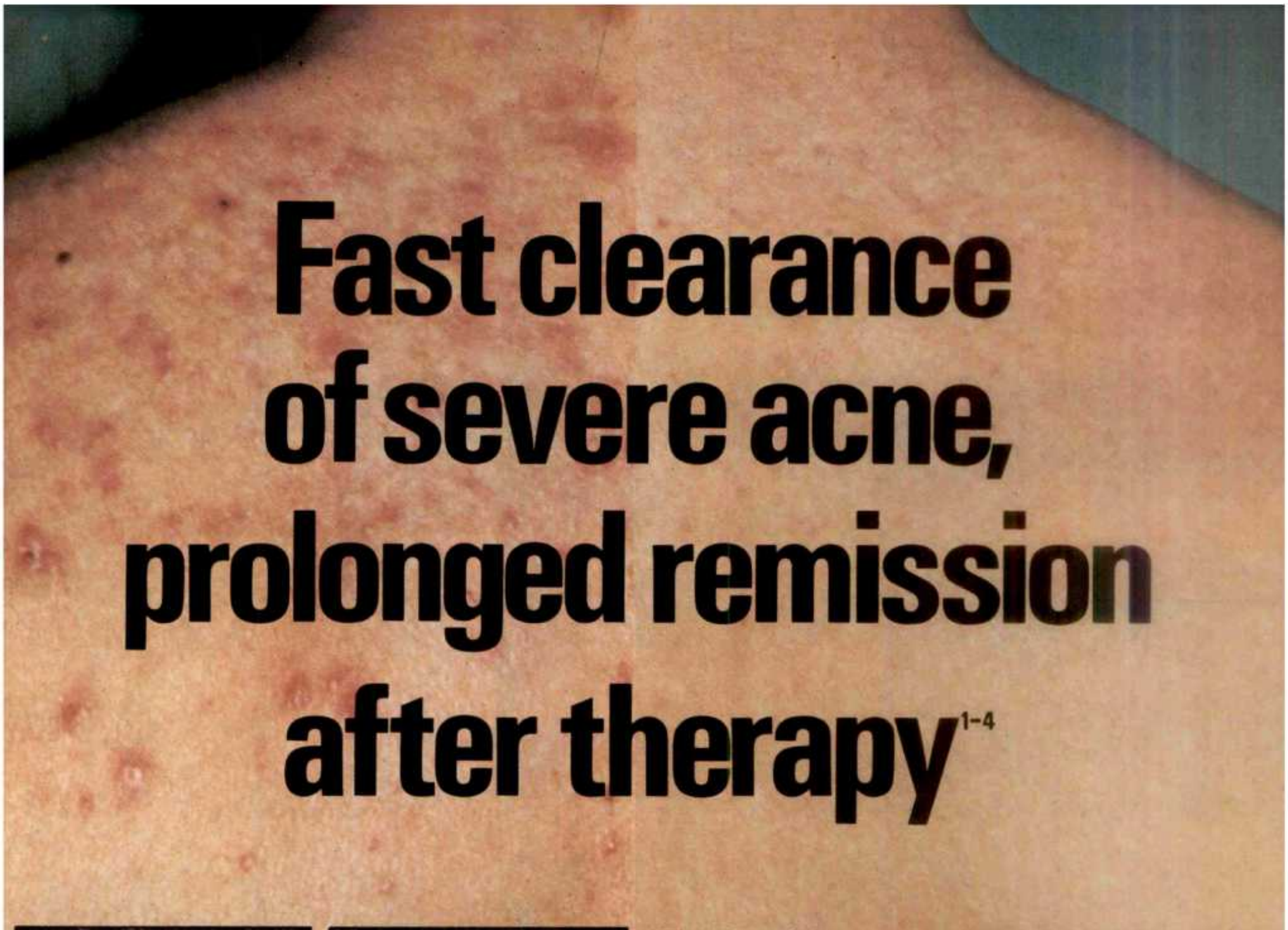

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(Main picture) Male patient with truncal acne unresponsive to oral antibiotics, before and after a course of Roaccutane.

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BRIEF PRESCRIBING INFORMATION

Indications Cystic and conglobate acne; severe acne unresponsive to systemic antibiotics.

Dosage 0.5mg/kg daily for 12-16 weeks. Increase up to 1mg/kg daily if response is poor at 4 weeks. Reduce dosage if tolerance is poor. As a rule, other anti-acne therapy, including antibiotics, is not indicated. Repeat course of treatment should not normally be given.

Contra-indications Pregnancy (Roaccutane is teratogenic – see 'Precautions') and breast-feeding. Hepatic or renal impairment.

Precautions The following precautions must be observed in women of childbearing potential.

1. Patients should be given detailed explanation of the risks.
2. Pregnancy should be excluded before therapy is instituted.
3. Effective contraception must be ensured for one month before treatment, during treatment, and for one month after treatment ends.

Use of patient information leaflets is strongly recommended. Monitor liver function and serum lipids (fasting value) regularly.

Side-effects Mainly mucosal dryness and fissures; facial dermatitis; desquamation; epidermal fragility; conjunctivitis; arthralgia/myalgia; increased hair loss (uncommon and reversible); possible elevation of liver enzymes and serum triglycerides. Benign intracranial hypertension has been reported, particularly in association with concomitant antibiotics.

Availability Hospitals and hospital clinics.

Presentations and Basic NHS Cost

Roaccutane capsules 5mg × 100 £33.56.

Roaccutane capsules 20mg × 100 £96.73.

Product Licence Numbers 0031/0158 (capsules 5mg), 0031/0160 (capsules 20mg).

Licence Holder

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PO Box 8, Welwyn Garden City
Hertfordshire AL7 3AY

References

1. In: "Retinoids: Advances in Basic Research and Therapy". Eds. Orfanos, C.E. *et al.* Springer-Verlag, Berlin, 1981, p. 219.
2. Brit. J. Derm., 1983, *108*, 333.
3. J. Amer. Acad. Derm., 1982, *6*, 735.
4. J. Amer. Acad. Derm., 1982, *6*, 766.
5. J. Amer. Acad. Derm., 1983, *9*, 629.

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Use: An aid in the topical treatment of venous and other types of skin ulcers.

Directions: Clean and dry the ulcer site, apply and smooth the Variclene gel over the affected area. Cover with a sterile dressing or pad and, where appropriate, apply compression bandaging. Repeat as required at intervals of not more than seven days.

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Presentation: 50g tube **Product Licence:** 0173/0015 (PA 278/10/1)

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Green aqueous gel containing 0.5% w/w Brilliant Green BP, 0.5% w/w Lactic Acid BP.

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British Journal of Dermatology (1984) **111**, 507–516.

Clinical and Laboratory Investigations

Dynamic changes in epidermal Ia-positive cells in allergic contact sensitivity reactions in mice

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SUMMARY

In mice sensitized with trinitrochlorobenzene, serial changes in epidermal Ia-positive cells were studied at various times after challenge. Until 3 days post-challenge, the Ia-positive cells consisted only of dendritic Langerhans cells; their number was decreased but they were significantly enlarged, with extending dendrites. Some Langerhans cells were also found surrounding a hair follicle, extending their dendrites toward the follicle like the spokes of a wheel. From 3 to 9 days after challenge, keratinocytes also began to express Ia antigens in the epidermis in addition to Langerhans cells, whose size diminished. This suggests that there are two phases in the response of the epidermal Ia antigens in contact sensitivity reactions, i.e. an early phase in which enlarged Langerhans cells are the only Ia-positive cells in the epidermis, and a late phase in which keratinocytes take over as the major Ia-positive cells, while Langerhans cells resume their original size. Ia antigen expression on keratinocytes in this late phase probably plays a crucial role in completely eliminating allergens deposited on the keratinocytes.

Ia antigens are demonstrable only on Langerhans cells (LCs) in the normal epidermis. Recently the expression of Ia antigens on the keratinocyte surface has been reported in several skin disorders in which cellular immune reactions are presumed to take place, i.e., graft-versus-host disease (Lampert *et al.*, 1982), lichen planus (Tjernlund, 1980), mycosis fungoides (Tjernlund, 1981; MacKie & Turbitt, 1983), psoriasis (Morhenn, Abel & Mahrle, 1982), and contact dermatitis (MacKie *et al.*, 1983). We have observed the same phenomenon in pityriasis rosea and spontaneous regressing flat warts (Aiba & Tagami, 1984). Experimental induction of Ia antigens on the keratinocytes was reported in graft-versus-host disease of rats (Lampert, Suitters & Chisholm, 1981; Mason, Dallman & Barclay, 1981) and mice (Daynes *et al.*, 1983; Breathnach & Katz, 1983). On the other hand, we still have little information as to the exact mode of the Ia antigen expression on the keratinocyte surface in delayed contact sensitivity, although Suitters & Lampert (1982) also recognized the expression of Ia antigen on the

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keratinocyte surface 3 days after the application of dinitrofluorobenzene (DNFB) to the ears of the sensitized rats.

Today the importance of LCs has been established in delayed contact sensitivity. Among epidermal cells LCs exclusively bear surface molecules with important immunological functions: C3 and Fc receptors (Stingl *et al.*, 1978) as well as Ia antigens (Rowden, Lewis & Delovitch, 1977; Stingl *et al.*, 1978; Rowden, Philips & Delovitch, 1978). LCs selectively take up small molecules, some of which serve as haptens in delayed contact hypersensitivity (Shelley & Juhlin, 1976). Moreover the surface density of LCs becomes a determining factor in the induction of sensitization or of hyporesponsiveness in delayed contact hypersensitivity (Towes, Bergstresser & Streilein, 1980; Streilein *et al.*, 1980). On the other hand, Nordlund & Ackles (1981a) reported that strong allergens such as DNFB cause a transient but definite decrease in the number of Ia-bearing cells following each sensitization and challenge.

The aim of the present work is to elucidate the kinetics of Ia-antigen expression on the keratinocytes after induction of delayed contact hypersensitivity concomitantly with the dynamic changes in density and morphology of epidermal Ia-positive LCs.

METHODS

Male mice [C3H/HeJ (H-2^k)], obtained from the Japanese Charles River Company (Kanagawa, Japan), were 9–13 weeks old when used. 2,4,6-Trinitrochlorobenzene (TNCB) was purchased from Sigma Chemical Company, St Louis, Missouri. Contact sensitization was induced with a small modification of a previously described method (Towes *et al.*, 1980; Streilein *et al.*, 1980). Briefly, mice were painted with 100 μl of 7% TNCB (4:1 acetone: olive oil) on the shaved dorsal body wall and the solution was air-dried. Five days after the epicutaneous painting, the ears were challenged with 50 μl of 1% TNCB in olive oil on each side of the ear.

As controls, before the sensitization and elicitation procedures, and at various times after the elicitation, groups of at least four mice were sacrificed. The thickness of the ears was measured using an engineer's micrometer (Mitutoyo, Tokyo, Japan), and the epidermis was separated for identification and enumeration of Ia-positive cells.

For the evaluation of the contact sensitivity, the ear swelling test was used according to Back & Larsen (1982). The ear swelling was expressed as a percentage according to the following formula:

$$\frac{\text{test ear} - \text{control ear}}{\text{control ear}} \times 100\ (\%)$$

Identification of Ia-positive cells

Because it gives better recognition of LCs and Ia antigens on the keratinocyte surface, immunofluorescence staining of epidermal sheets was performed according to the technique of Nordlund *et al.* (1981b). Briefly, mice were killed and their ears were amputated at the base. The four edges of the ears were cut away in 2–4 mm widths and the central portions of them were cut into small 4 to 6 mm square pieces. These pieces were incubated for 2·5 h in 20 mM EDTA in phosphate-buffered saline (PBS) at 37°C. After incubation, they were pressed on to the adhesive surface of Scotch tape and the adhered skin pieces were then peeled off from the dermis with forceps. Only the epidermis obtained from the dorsal surface of the ear was used. These epidermal sheets were washed twice in PBS, fixed in absolute ethanol for 20 min at 4°C, rehydrated in PBS, and incubated in 2% bovine serum albumin (Sigma) in PBS for 30 min at 37°C. Then they were treated in a 1:100 dilution in PBS of anti-Ia^k monoclonal antibody

(Becton Dickinson, Facs. Systems, Sunnyvale, California), washed three times in PBS, and incubated in a 1:40 dilution in PBS of fluoresceinated rabbit anti-mouse IgG (whole molecules, Miles-Yeda Ltd Research Products, Israel). After an additional three washes in PBS, the pieces were mounted on slides in PBS/glycerol under a coverslip and examined with a Nikon fluorescence microscope. The use of tape backing facilitated the separation and transfer of the very thin mouse epidermal sheets while concurrently holding the sheets flat for exposure to fixatives and staining solution; it did not disturb the observation by the fluorescence microscope.

Enumeration of Ia-positive dendritic cells

More than 500 Ia-positive dendritic cells from each mouse were counted in more than five randomly chosen areas of at least three specimens under the fluorescence microscope with a graticule fitted into the eyepiece. Cell populations were reported as the total number of Ia-positive cells per mm^2 skin ± s.e.m.

Measurement of the size of Ia-positive dendritic cells

In each mouse of each group, randomly selected fields were photographed at a magnification of 400 with Fujichrome 100 film for colour slides (Fuji Photo Film Co., Ltd, Tokyo, Japan). The slides were projected over L-A4 copying paper (Fuji Xerox Co., Ltd, Tokyo, Japan) with a Pradovit 250 colour slide projector (Ernst Leitz, GmBh, F.R.G.) and the outlines of Ia-positive dendritic cells were traced with a pencil including their dendritic processes. These were cut out from the paper with a razor and their weight was measured using a AE 160 electron microbalance (Mettler Instrument AG, Greifensee, Switzerland). Changes of cell size were expressed as a ratio to normal control ± standard error of mean.

Student's *t*-test was used to assess differences among groups. A *P* value of less than 0·01 was considered significant. At least four animals were used in each experimental group.

RESULTS

Epicutaneous application of 7% TNCB (100 μl in 4:1 acetone:olive oil) to the back of C3H mice induced sensitization in all of the animals. The time course of the response after challenge was assessed with the ear swelling test (Fig. 1). A significant increase in ear swelling was observed between 12 h and 7 days ($P < 0{\cdot}01$), with a maximum response at 24 h.

Challenge with 1% TNCB on the ear resulted in a decrease of the surface densities of Ia-positive LCs from 6 h to 48 h. This was followed by a gradual increase, reaching a plateau 7 days after challenge (Fig. 2). A statistically significant decrease was observed at 6, 12, and 48 h, and a significant increase at 7 days post-challenge respectively ($P < 0{\cdot}01$).

The change in the size of Ia-positive LCs after challenge is shown in Fig. 3. Compared with the LCs in non-sensitized mice (Fig. 4), those observed in epidermal sheets showed a significant increase in size at 6, 12 (Fig. 5), 24 (Fig. 6) and 48 h after challenge ($P < 0{\cdot}01$), with a maximum enlargement at 12 h. After 72 h, their size began to decrease, returning to the control range. It was observed that LCs extended their dendrites for 3 days after challenge. In addition, from 6 h to 48 h, we unexpectedly found that in a localized area of the epidermis, some LCs appeared to surround a hair follicle, extending their dendrites toward the follicle (Fig. 6).

Keratinocytes were observed to express Ia antigen on their cell surface for a certain period of time after challenge with 1% TNCB (Fig. 7). The time-course study of the expression of Ia antigen on the keratinocyte surface demonstrated that Ia antigen became recognizable on the

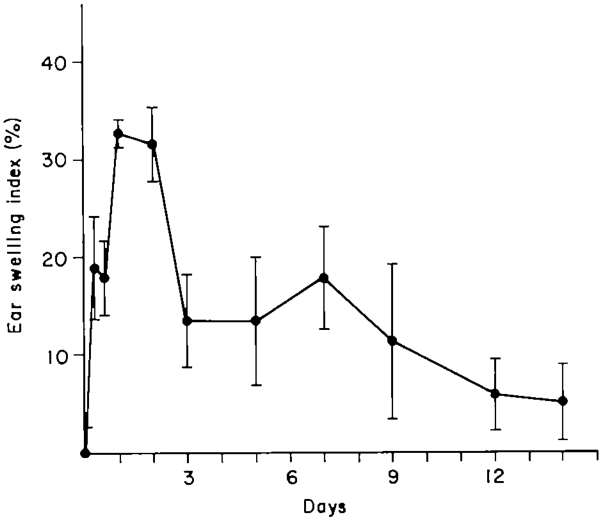

FIGURE 1. Time course of ear swelling index after challenge. Mean ± s.e.m. percentage increase in ear swelling index is shown.

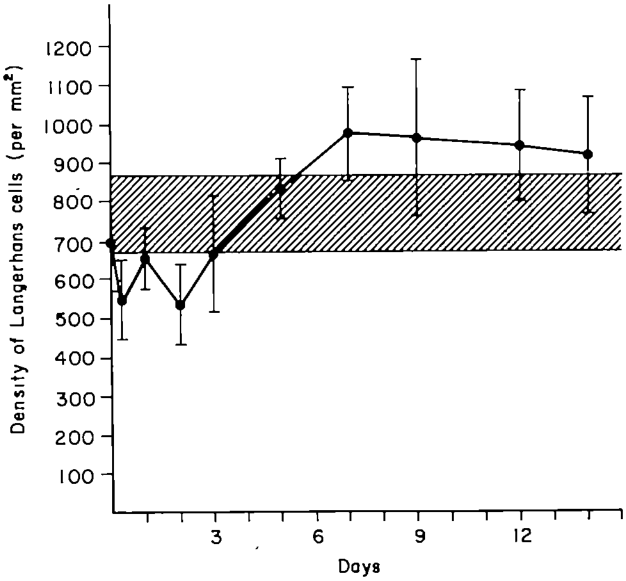

FIGURE 2. Time course of the density of Langerhans cells after challenge. Mean ± s.e.m. of number of Langerhans cells in mm² of the epidermal sheet is shown. Shaded area indicates normal range (mean ± s.e.m.).

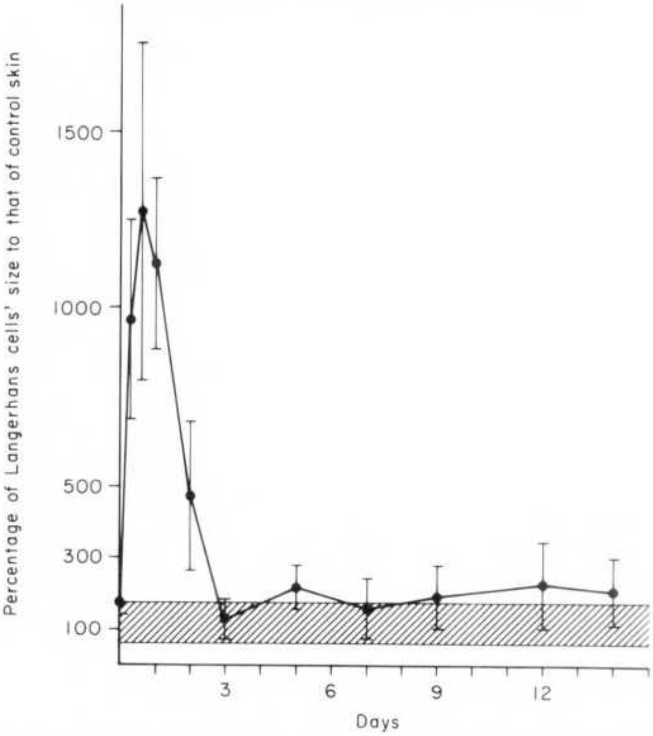

FIGURE 3. Change of the size of Langerhans cells after challenge. Mean size of Langerhans cells as compared with that of normal controls ± s.e.m. is shown. Shaded area indicates normal range (mean ± s.e.m.).

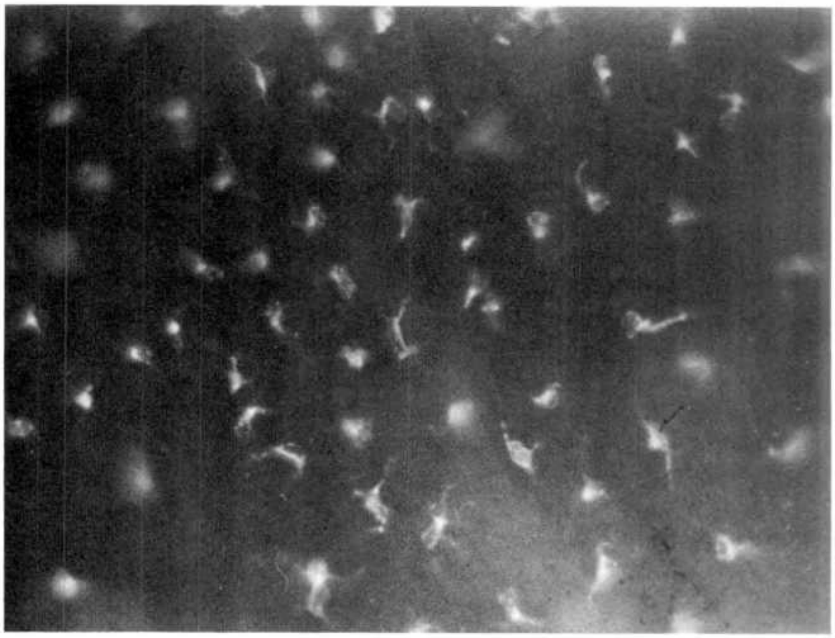

FIGURE 4. Ia-positive dendritic Langerhans cells on the epidermal sheet in the non-sensitized mouse (× 400).

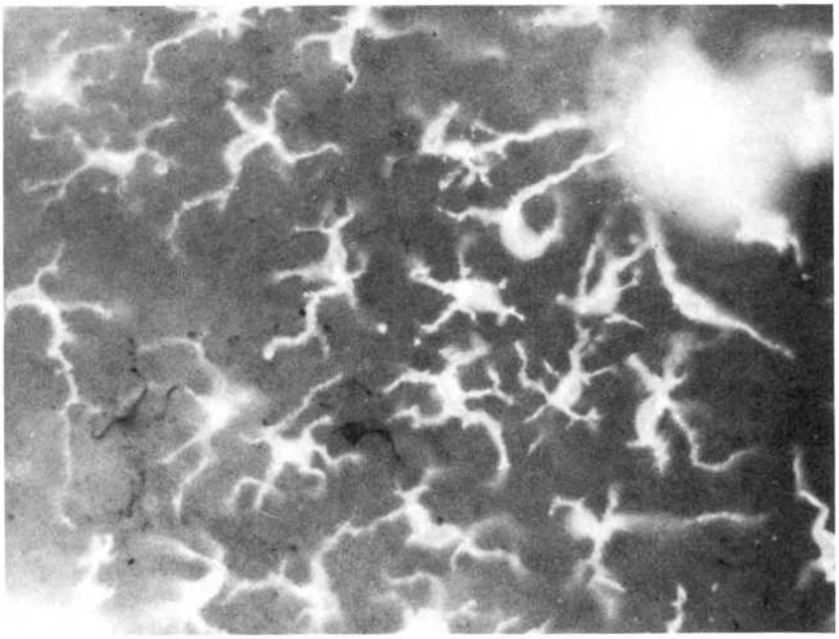

FIGURE 5. Large Ia-positive dendritic cells on the epidermal sheet in the mouse 6 h after induction (× 400).

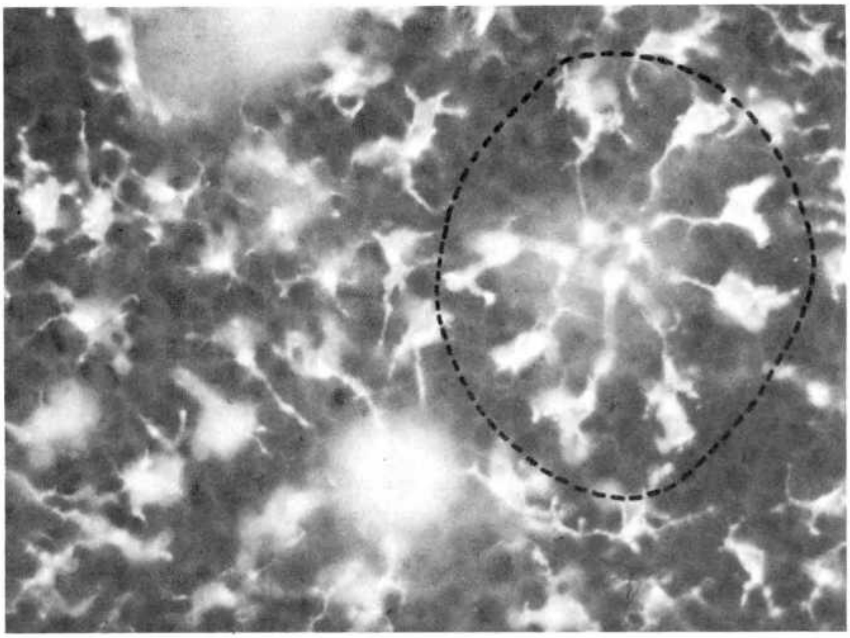

FIGURE 6. Large Ia-positive dendritic cells, surrounding a hair follicle like a wheel (circled area), on the epidermal sheet in the mouse 24 h after challenge (× 400).

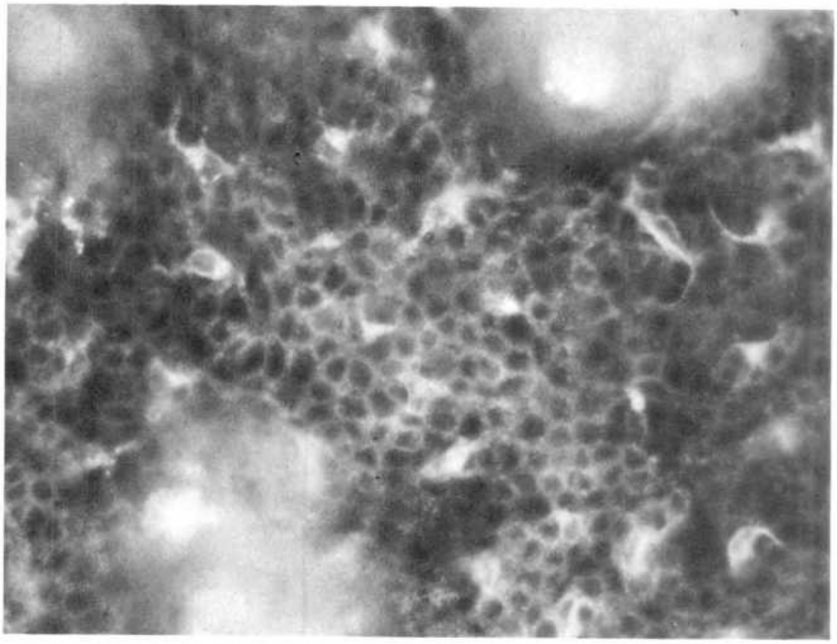

FIGURE 7. Ia antigen expression on the keratinocytes in the epidermal sheet of the mouse 72 h after challenge, recognized as epidermal intercellular staining with anti-Ia antibody (× 400).

TABLE 1. Time course of Ia antigen expression on the keratinocyte after challenge

Expression of Ia antigens on the keratinocytes	Time post-challenge											
	C[a]	0 h[b]	6 h	12 h	24 h	48 h	72 h	5 d	7 d	9 d	12 d	14 d
No. of mice positive / No. of mice challenged	0/6	0/4	0/8	0/4	0/8	1/8	8/8	8/8	5/5	5/5	1/4	0/4

[a] Non-sensitized mice.

[b] Mice 5 days after sensitization, without induction.

keratinocyte in the localized area of the epidermis in all the specimens from 72 h to 9 days post-challenge (Table 1).

DISCUSSION

In TNCB-sensitized mice the surface density of Ia-positive LCs first decreased until 3 days post-challenge, but then increased to reach a peak after 7 days. This is in contrast to the findings of Nordlund & Ackles (1981a) and Gschnait & Grenner (1979). In their study with C3H mice sensitized with DNFB, Nordlund & Ackles (1981a) found that Ia-positive LCs increased transiently at 24 h but decreased significantly for 48 to 120 h post-challenge. They sensitized mice twice with 50 μl of 0·5% DNFB in acetone–corn oil on the left ear and shaved abdominal wall and challenged twice, 4 and 7 days later, with 50 μl of 0·2% DNFB on the opposite right ear. On the other hand, Gschnait & Grenner (1979) reported that they could not find any significant decrease in the surface density of leucine-aminopeptidase (LAP)-positive LCs of the flanks during 8 days of treatment with 0·25% DNCB twice daily. We are not sure whether these differences in the results of the experiments are simply due to differences in methods.

In contrast to their decreased number for 3 days after challenge, LCs increased in size significantly, extending their dendrites actively. It has been reported in delayed contact sensitivity that LCs show signs of increased activity in their cytoplasm, as shown by numerous multivesicular bodies, prominent Golgi regions and endoplasmic reticulum, and numerous Langerhans granules in the early hours after antigen challenge, while later in the reaction, some LCs show signs of damage (Silberberg-Sinakin & Thorbecke, 1979). Giant LCs were also induced by psoralen and UV-A irradiation (PUVA) (Juhlin & Shelley, 1979); these PUVA-induced giant LCs were small in number, showing no prominent dendrites. It is not clear whether the enlarged LCs observed by us in contact allergic reactions are the cells that correspond to those showing active ultrastructural changes or whether they have the same biological functions as the PUVA-induced giant LCs.

It is of note that during 6 to 24 h post-challenge, the enlarged LCs surrounded a hair follicle, extending their dendrites toward the follicle. We think that such a peculiar arrangement of LCs suggests an important role played by the hair follicles as a shunt pathway for permeation of contact allergens through the skin, i.e., the follicular epithelium probably contains a much greater amount of antigen than the surrounding epidermis which induces elongation of the dendrites of LCs toward it.

We found that Ia antigen expression occurs on the keratinocyte surface for a prolonged period, from 3 days to 9 days after challenge. Furthermore, as Ia antigens become recognizable on the keratinocyte surface, Ia-positive LCs decrease in size as compared with those noted until 72 h post-challenge. Although the Ia antigen expression on the keratinocytes was easily detected in a horizontal place, in epidermal sheets, we note that it became much less obvious when observed in a cryostat specimen prepared in a vertical section, being chiefly present in the lower portions of the epidermis. Suitters & Lampert (1982) noted the Ia antigen expression on the keratinocyte surface of rats only in delayed contact sensitivity, but not in croton oil irritant dermatitis. Based on our observation, we think that the coexistence of these smaller Ia-positive LCs and Ia-positive keratinocytes is a feature that indicates the active role played by the latter in delayed contact sensitivity. We still do not have any data on the origin of Ia antigens on the keratinocytes in delayed contact sensitivity, although Breathnach & Katz (1983) demonstrated the synthesis of Ia antigens by keratinocytes in acute cutaneous graft-versus-host disease in mice.

Our data strongly suggest that contact sensitivity reactions can be divided into two phases from the distribution pattern of Ia antigens in the epidermis, viz., an early phase until 3 days after challenge when enlarged LCs are the only epidermal Ia-positive cells, possibly playing a major immunological role, and the late phase from 3 to 9 days when keratinocytes also participate in the reaction as major Ia-positive cells. It seems likely that allergens that are initially present on the LCs elicit an acute inflammatory reaction, but later, when LCs resume their original small size, because of the removal of allergens from their surface, those allergens localized on the keratinocytes also provoke prolonged inflammatory changes, until there is complete elimination of any residual allergens from the epidermis. With regard to the mechanism for induction of Ia antigens on the keratinocyte surface, we think that T-cell-soluble factors such as those involved in the expression of Ia antigen on Ia-negative macrophages

(Steinman *et al.*, 1980; Steeg, Moore & Oppenheim, 1980; Calamai, Beller & Unanue, 1980), B lymphoma (Kim *et al.*, 1983) and vascular endothelium (Pober *et al.*, 1983) are responsible.

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Factors affecting the binding of lectins to normal human skin

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SUMMARY

Factors affecting the binding of a wide range of lectins to normal human skin were examined in order to evaluate current discrepancies in the literature. The profile of specific binding characteristic for each lectin was found to be variously influenced by the source of conjugate, tissue-processing method, the effectiveness of saccharide inhibitors, and by individual and minor body site variations. Most significantly, the use of routine histological processing not only greatly reduced binding intensity overall but also altered the binding pattern.

Lectins are a group of proteins which have the property of binding to certain specific sugar chain structures including those of glycoproteins (Debray *et al.*, 1981), glycolipids (Zurn, 1982) and glycosaminoglycans (Toda *et al.*, 1981). As these three classes of glycoconjugate are present at the cell surface and are believed to play important roles in the interactions of cells with their environment (Hynes, 1979), lectins are now widely used as tools to investigate the cell surface and how it is modified by differentiation, malignant transformation and the presence of genetic defects (Brown & Hunt, 1978).

In spite of their obvious potential in the study of normal epidermal differentiation and the aetiology of skin diseases there have been relatively few reports of the application of lectins to skin biology. In addition, the available reports are in some disagreement as to the lectin binding profile of normal human epidermis. For example Reano *et al.* (1982) report that normal human epidermis does not label with *Bandeiraea simplicifolia* lectin I (BSA I), *Ulex* lectin (UEA I) or wheat germ agglutinin (WGA) whilst Nemanic, Whitehead & Elias (1983) obtained labelling with UEA I and WGA and Bell & Skerrow (1983) obtained labelling with all three lectins. Also whilst most authors are agreed that Con A binds to viable epidermal cells in frozen sections, Louis *et al.* (1981) found no reaction in a retrospective study of paraffin sections. Possible explanations of these discrepancies may lie in the different lectin preparations and tissue-processing techniques used by the authors but currently no study has been performed on the optimizing of lectin binding methodology.

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It is clearly essential to establish consistent information about lectin binding to human skin before deviations from normal can be recognized or interpreted. This paper therefore presents a detailed study of factors affecting the reaction of a wide range of lectins with normal human skin. In particular the effect of routine histological processing on lectin binding sites is investigated to examine the possibility of obtaining data retrospectively from routine files.

METHODS

General biochemicals and enzymes were obtained from Sigma (Poole, Dorset, U.K.). Chemicals were of analytical grade and were purchased from BDH (Poole, Dorset, U.K.). Fluorescein isothiocyanate (FITC) and tetramethylrhodamine isothiocyanate (TRITC)-labelled lectins were purchased mainly from E-Y Laboratories Inc. (San Mateo, California, U.S.A.) and also from Vector Labs Inc. (Burlingham, California, U.S.A.) and Sigma as shown in Table 1.

TABLE 1. Lectins used for histochemical staining. Con A, LCH and WGA can bind to internal as well as terminal saccharide residues

Source	Common name/ abbreviation	Major carbohydrate specificity	Saccharide inhibitor	Supplier
Concanavalin ensiformis	Con A	αD-mannosyl αD-glucosyl	αmethyl-mannoside	Sigma, E-Y
Lens culinaris	LCH	αD-mannosyl αD-glucosyl	αmethyl-mannoside	Sigma, E-Y
Bandeiraea simplicifolia	BSA II	*N*-acetylglucosaminyl	*N*-acetylglucosamine	E-Y
Triticum vulgaris	Wheat germ agglutinin: WGA	(β*N*-acetylglucosaminyl)n *N*-acetylneuraminyl	*N*,*N*′diacetylchitobiose	E-Y
	Succinylated WGA	(β*N*-acetylglucosaminyl)n	*N*,*N*′diacetylchitobiose	Vector
Arachis hypogaea	Peanut lectin PNA	βD-galactose(1-3)-*N*-acetylgalactosaminyl	D-galactose	E-Y
Bandeiraea simplicifolia	BSA I	D-galactosyl	D-galactose	Vector, E-Y
Glycine max	Soy bean lectin: SBA	*N*-acetylgalactosaminyl	*N*-acetylgalactosamine	E-Y
Helix pomatia	HPA	α*N*-acetylgalactosaminyl	*N*-acetylgalactosamine	E-Y
Ulex europaeus	UEA I	L-fucosyl	L-fucose	E-Y
Limulus polyphemus	LPA	*N*-acetylneuraminyl	*N*-acetylneuraminic acid	E-Y

Tissue preparation

Skin samples were obtained during routine surgery from a variety of body sites including foreskin, abdomen, breast, leg and forehead, and snap frozen with or without O.C.T. embedding compound (Lab-Tek Products Division, Miles Laboratories Inc., Naperville, U.S.A.) directly on to chucks pre-cooled in dry ice/acetone slush. Specimens were stored at −20°C until required. Six to eight micron sections were cut on a cryostat at −25°C as required, collected on gelatin-coated slides and air-dried for 15–30 min. Formalin-fixed paraffin-embedded skin biopsies were obtained from the routine files of the Department of Dermatology, University of Glasgow. Six micron sections were cut, deparaffinized in xylene, rehydrated and rinsed in appropriate buffer before application of lectin conjugate.

Choice of lectin conjugates

Of the hundreds of known lectins about forty are commercially available from various chemical supply houses purified and suitably labelled for histochemical investigations. A range of these were chosen to include the major lectin groups as defined by carbohydrate binding specificity and to compare with the existing literature (Table 1).

Application of lectin conjugates to tissue sections

On the day of use, lectin conjugates were diluted to 100 μg/ml (FITC) or 200 μg/ml (TRITC) in appropriate buffer and *c.* 100 μl applied to the sections for 30 min at room temperature in a humidified chamber. The lectin was then rinsed off and the sections washed with stirring in a large volume of appropriate buffer for 15 min before being mounted in buffered glycerol or UV-inert mountant (Gurr). Appropriate buffers are as follows: for BSA I, BSA II, HPA, PNA, SBA, UEA I, WGA and sWGA Dulbecco's PBS pH 7·2; for LPA 0·05 M Tris–HCl, 0·01 M $CaCl_2$, 0·1 M NaCl pH 7·8; and for Con A and LCH 0·05 M Tris–HCl, 0·01 M $CaCl_2$, 0·01 M $MnCl_2$, 0·1 M NaCl pH 7·2. (The $MnCl_2$ was added to this buffer just before use to avoid precipitation.) Competitive saccharide inhibition controls were run routinely using the inhibitors shown in Table 1. For these one volume of lectin at twice the required concentration was diluted with one volume of 0·3 M saccharide in 0·05 M Tris buffer pH 7·2 and left for an hour at room temperature before use. *N,N*-diacetylchitobiose was used at a concentration of 20 mg/ml in PBS. Sections were also incubated with saline alone as an autofluorescence control.

Fluorescence microscopy

Specimens were examined with a Leitz Orthoplan microscope equipped with a Ploemopak 2·1 fluorescence illuminator fitted with an I_2 filter block for FITC and a N_2 block for TRITC. Photographs were taken with a Leitz Orthomat automatic camera. Best results were obtained with Ilford XP1 400 film rated at 1600 ASA.

RESULTS

Conditions for lectin labelling—general findings

Preliminary experiments determined that: (1) lectin concentrations giving the maximum fluorescence consistent with good resolution were in the order of 100 μg/ml for FITC-conjugated lectins and 200 μg/ml for TRITC conjugates applied for 30 min at room temperature; (2) lectin-binding patterns were unchanged by sample storage at − 20°C for at least several months; (3) O.C.T. embedding compound did not improve the results; and (4) better results were generally obtained with TRITC conjugates, as non-specific fluorescence and fading were reduced and autofluorescence was less with the N_2 filter block.

A major difficulty encountered was the inactivity of some of the lectin preparations, presumably due to incorrect handling during transit which was usually of necessity trans-Atlantic. This was a particular problem with certain lectins. Two out of four batches each of Con A and LCH and all four batches of LPA were found to be inactive when tested against known positive substrates with appropriate controls. Marked non-specific fluorescence was noted in some FITC conjugates [BSA I (E–Y but not Vector) and UEA I] suggesting the presence of free FITC or inactive conjugate.

Patterns of lectin binding to unfixed frozen skin sections

The lectins shown in Table 1 were applied to approximately twenty samples from various body

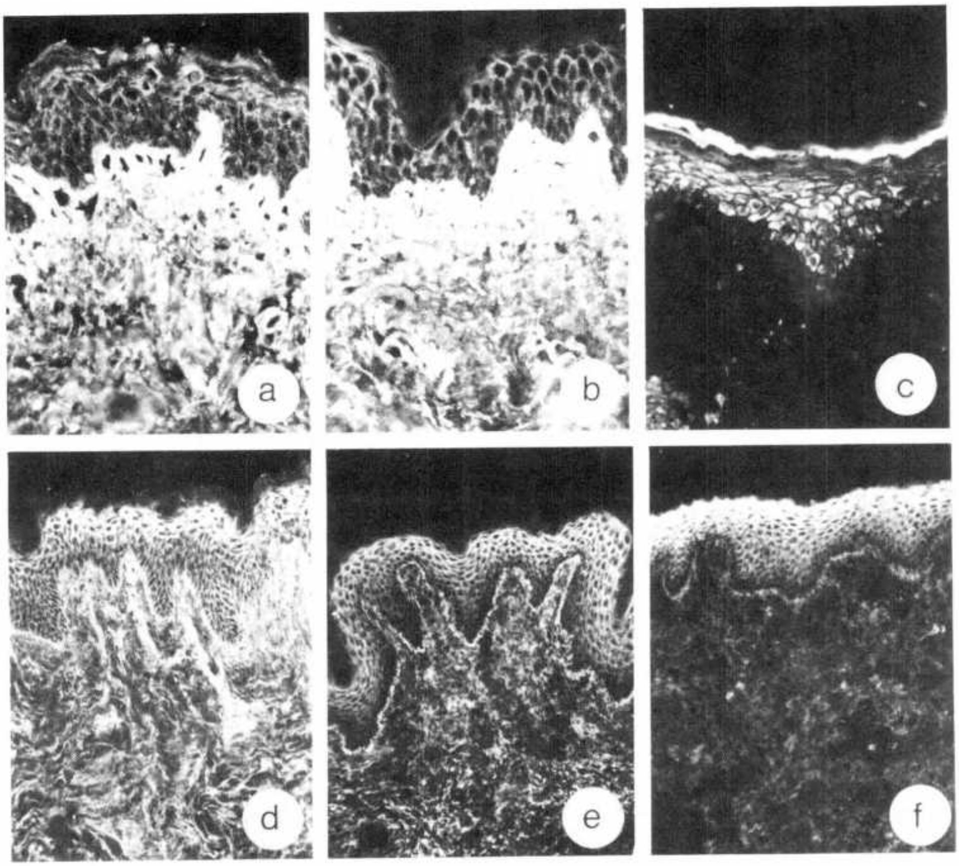

FIGURE 1. Patterns of lectin binding to frozen sections of unfixed normal human skin, each lectin showing a characteristic profile of intensity and distribution. The staining is specific except for that of the stratum corneum in a and c. (a) Con A, breast, (magnification, × 350). (b) LCH, abdomen, × 350. (c) BSA II, forehead, × 350. (d) WGA, foreskin, × 140. (e) WGA blocked with *N*-acetylglucosamine, foreskin, × 140. (f) sWGA, foreskin, × 140. (g) PNA, foreskin, × 140. (h) BSAI, foreskin, × 140. (i) SBA, breast, × 350. (j) HPA, foreskin, × 350. (k) UEA I, foreskin, × 140. (l) UEA I, foreskin, × 410.

sites including foreskin, abdomen, breast, leg and forehead. Each lectin gave a characteristic pattern of intensity and distribution with only minor variation between body sites (Fig. 1). In general both epidermal and dermal components were labelled but the stratum corneum did not react specifically with any of the lectins. Labelling of epidermal cells appeared peripheral with most lectins, and as glycoconjugates are mainly localized at the cell surface, the staining is assumed to be intercellular. Cytoplasmic staining was observed with Con A, BSA II and in some samples with UEA I. Unless specifically discussed below, staining was completely abolished in the saccharide controls (Table 1).

Individual lectins

Concanavalin A (αD-mannosyl/αD-glycosyl, Fig. 1a) reacted with both dermis and epidermis. Both the cytoplasm and periphery of the epidermal cells were stained. This is consistent with the

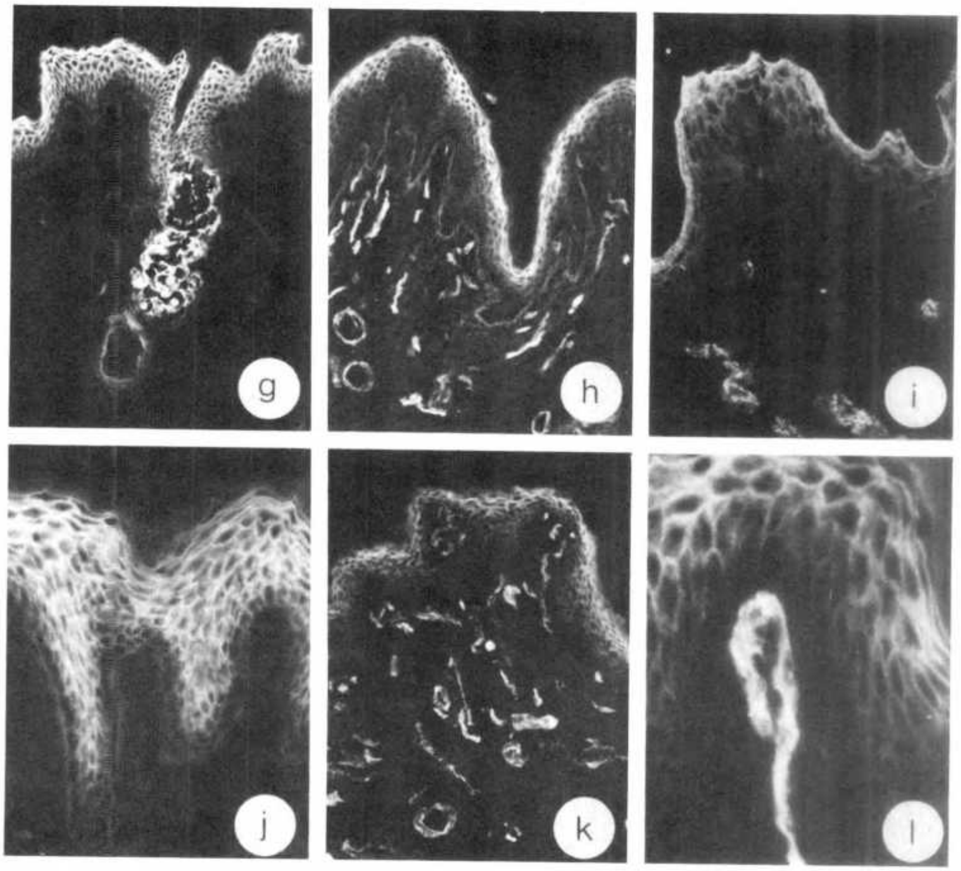

FIGURE 1. (Cont.)

ability of Con A to bind to core oligosaccharides. In addition, perinuclear fluorescence was sometimes observed. The dermis reacted particularly intensely, presumably largely due to the glucose residues of collagen. Apart from the stratum corneum the reaction was inhibited by freshly dissolved α-methyl mannoside.

Lens culinar's agglutinin (αD-mannosyl/αD-glucosyl, Fig. 1b). LCH which has a similar monosaccharide specificity to Con A displayed a similar overall reaction pattern. However, the reaction was only partially inhibited by α-methyl mannoside.

Bandeiraea simplicifolia lectin II (N-acetylglucosaminyl, Fig. 1c). The characteristic binding pattern of this lectin was a faint intracellular staining of the mid-epidermal region mainly in and around hair follicles and sebaceous glands. The stratum corneum reacted non-specifically. Dermal capillaries were also visualized in some samples. BSA II is known to interact with glycogen (Goldstein & Hayes, 1978) and the distribution of BSA II staining in epidermis resembles that of glycogen (Freinkel, 1983). Digestion with 10 U/ml α-amylase for 30 min at room temperature prior to application of lectin abolished BSA II staining supporting identification of glycogen as the BSA II cytoplasmic binding site.

Wheat germ agglutinin [β(N-acetylglucosaminyl)n/N-acetylneuraminyl, Fig. 1d]. WGA binds specifically to *N*-acetylglucosamine and its β 1–4-linked oligosaccharides and to *N*-acetylneuraminic (sialic) acid probably at the same binding site (Monsigny *et al.*, 1980). WGA reacted strongly with the viable epidermis, the basement membrane zone and the dermis. The reaction was blocked slightly by *N*-acetylneuraminic acid (a weak inhibitor), more strongly by *N*-acetylglucosamine (a more effective inhibitor), especially in the dermis and basal layer (Fig. 1e), and completely abolished by *N*,*N*′-diacetylchitobiose, a strong inhibitor of WGA.

Succinylated WGA [β(N-acetylglucosaminyl)n, Fig. 1f]. sWGA is thought to be unable to bind to *N*-acetylneuraminic acid although its reaction with *N*-acetylglucosamine appears similar to WGA (Monsigny *et al.*, 1980). sWGA reacted with the dermis and suprabasal epidermal cells. The dermal reaction was noticeably less intense than with WGA and the basement membrane zone generally did not react. Except for a faint fluorescence at the level of the granular layer the reaction was abolished by *N*,*N*′-diacetylchitobiose but only slightly diminished by the weaker inhibitor *N*-acetylglucosamine. Taken together the binding patterns of WGA and sWGA suggest that the interaction of WGA with basal cells and the basement membrane zone involves sialic acid residues as does some of the dermal reaction. This result is consistent with reports that various lectin binding sites are masked by sialic acid in basal cells (Nemanic *et al.*, 1983) and with the finding that basal cells have a higher sialic acid content of their biantennary glycoproteins than differentiated cells (Roelfzema & van Erp, 1983).

Peanut lectin [βD-galactose(1-3)-N-acetylgalactosaminyl, Fig. 1g]. An intense intercellular staining was observed in the viable epidermis with PNA, although basal cells reacted very weakly. Sebaceous gland cells were also strongly outlined. In some samples a band of fluorescence was apparent at the basement membrane zone. This was especially evident in forehead skin.

Bandeiraea simplicifolia lectin I (D-galactosyl, Fig. 1h). This lectin reacted most intensely with the granular layer as well as the basement membrane zone, dermal capillaries and sebaceous glands. The intensity of the granular layer stain was variable between individuals, being strong in *c.* 20% of the samples, moderate in 40%, weak in 20% and very weak/absent in 10%. This variation was seen even when samples from similar body sites were stained with the same lectin solution suggesting individual variation in BSA I binding sites. This may reflect the blood group of the donor as ABH isoantigens are expressed in the granular layer of normal skin (Szulman, 1960) and BSA I exhibits specificity for blood group B (Goldstein & Hayes, 1978). The basement membrane reaction may be due to laminin which is known to bind BSA I (Shibata *et al.*, 1982).

Soy bean agglutinin (N-acetylgalactosaminyl, Fig. 1i). SBA outlined the suprabasal layers of the viable epidermis with a gradual increase in intensity with the level of differentiation. Patches of granular fluorescence were also observed in the dermis, frequently outlining vessels.

Helix pomatia agglutinin (αN-acetylgalactosaminyl, Fig. 1j). HPA bound to the viable epidermis, the basement membrane zone and to dermal capillaries. The reaction with the basal layer was weak.

Ulex europaeus agglutinin (L-fucosyl, Fig. 1k). UEA preferentially stained the upper viable epidermis with cells of hair follicles reacting particularly intensely. Cytoplasmic fluorescence was apparent in some samples. Individual variation in reaction intensity was observed. It is possible that this is a blood group effect as UEA I has blood group O specificity, though Holthoffer *et al.* (1982) have reported UEA I staining of epithelial structures to be independent of blood group and secretor status. A notable feature of this lectin is its ability to outline dermal capillaries, in agreement with the presence of UEA I binding sites on vascular endothelium (Holthoffer *et al.*, 1982). Capillary staining was observed even when the epidermal reaction was faint and the high resolution of the stain suggests that UEA I may be a valuable tool in the investigation of dermal microvasculature especially if further studies allow a distinction between lymphatic and capillary channels to be made.

Limulus polyphemus agglutinin (N-acetylneuraminyl). No reaction of LPH with either epidermis or dermis could be demonstrated in agreement with previous reports (Reano *et al.*, 1982; Nemanic *et al.*, 1983). However, as the preparation of this lectin appeared slightly aggregated it was tested for activity against fresh horse erythrocytes both by haemagglutination and examination of cell surface fluorescence. No specific activity could be demonstrated in either this or three replacement preparations. Thus, it cannot be concluded at present whether or not this sialic acid binding lectin reacts with skin.

Patterns of lectin binding in acetone-fixed frozen skin sections

The impression was gained consistently that the brightness and resolution of fluorescence was slightly increased when frozen sections were fixed in acetone at −20°C for 10 min prior to staining, presumably due to the precipitating effect of this fixative. With the exception of a loss of the granular dermal staining observed with SBA the staining patterns were identical.

Patterns of lectin binding in deparaffinized formalin-fixed skin sections

The most striking difference was a marked reduction in fluorescence intensity so that at first sight the results often appeared negative. The photographs in Fig. 2 only appear as intense as in Fig. 1 because they received a much longer exposure time. This seemed largely due to the formalin, since fixation of frozen sections for as little as 2 min dramatically reduced lectin binding. Autofluorescence was greatly increased in some samples and inconsistent patterns were occasionally observed. In addition, the binding patterns differed from those seen in unfixed material. In contrast to frozen sections, specific binding was not observed with SBA, sWGA, BSA I and UEA I, even in specimens strongly reactive with the latter two lectins when unfixed. Specific binding was only observed with BSA II when the reaction observed with frozen sections of the same material was very strong. Con A, HPA, LCH, PNA and WGA bound specifically to deparaffinized sections but the binding patterns differed from those of unfixed sections as follows: with Con A and LCH dermal staining was reduced especially in the reticular dermis and perinuclear staining was not observed (Fig. 2a, b); WGA no longer bound to the basement membrane zone and no increase in intensity with level of differentiation was observed (Fig. 2c); with HPA, PNA and WGA blocked with *N*-acetylglucosamine the sparing of the basal layer was hardly apparent and again no basement membrane zone reaction or increase in intensity with differentiation was seen (Fig. 2d–f). In addition sebaceous gland staining was no longer apparent with PNA and a much greater reduction in intensity occurred when WGA was blocked with *N*-acetylglucosamine.

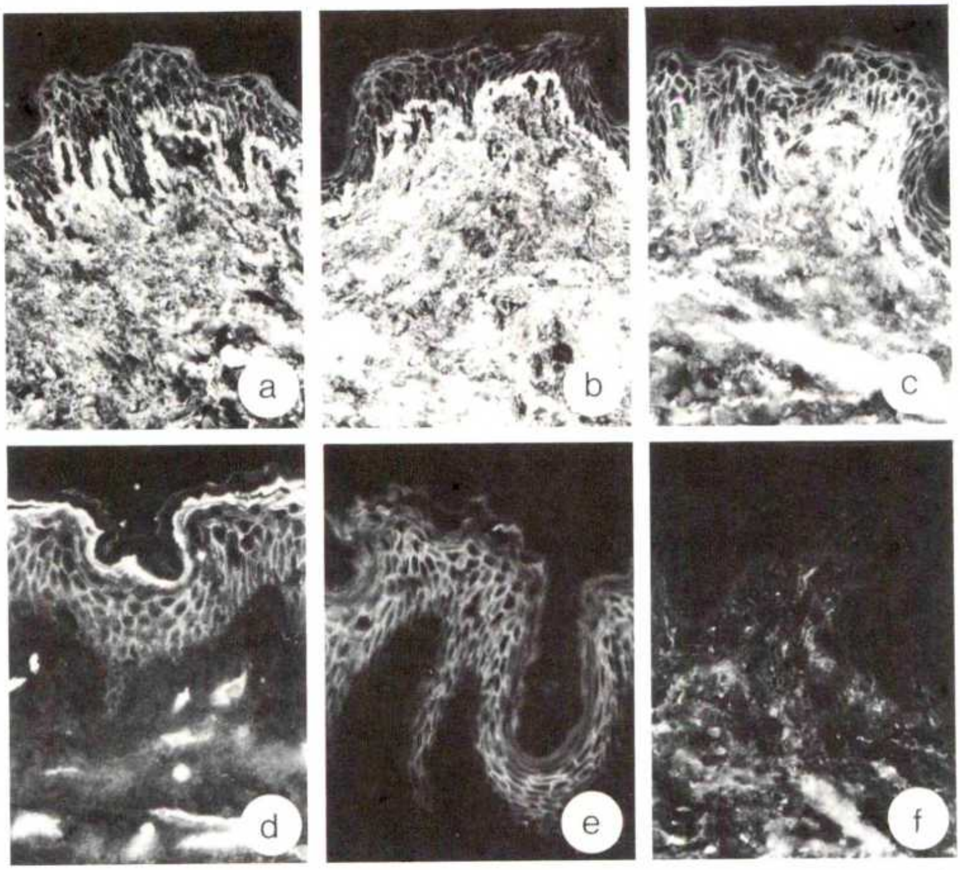

FIGURE 2. Patterns of lectin binding to formalin-fixed, paraffin-embedded sections of normal human skin showing marked alterations to the unfixed patterns. Due to the weakness of fluorescence these photographs required much longer (*c.* × 5) exposure times than those in Fig. 1. (a) Con A, breast. (b) LCH, breast. (c) WGA, breast. (d) HPA, foreskin. (e) PNA, foreskin. (g) WGA blocked with *N*-acetylglucosamine, breast. Final magnifications, all × 350.

DISCUSSION

It is apparent from these results that though lectins constitute a series of differentiation markers for keratinocytes, the observed lectin binding patterns are sensitive to a variety of experimental conditions and can even show variation between individuals. Thus, in order to obtain reproducible and meaningful results the methods must be carefully considered and controlled.

Firstly, the activity of each lectin preparation must be checked against a known positive substrate especially if a negative result is obtained. The difference spectroscopy test suggested by Rudiger (1983) may facilitate this. Also a fully effective saccharide inhibitor must be chosen to avoid false identification of non-specific reactions. The finding that the WGA and sWGA reactions could be inhibited by the disaccharide *N,N′*-diacetylchitobiose but not by *N*-acetylglucosamine, a commonly used monosaccharide inhibitor of WGA, may explain the failure of some authors to find specific binding with WGA.

Secondly, it must be appreciated that tissue preparation method can markedly affect the observed lectin binding patterns. From the results of this study it appears that the mild precipitating fixative, acetone, applied at −20°C has little effect on epidermal binding sites though some dermal sites were destroyed, most probably by extraction. In contrast, as might be expected by its ability to cross-link, denature, extract and chemically modify tissue components (Pearce, 1980), significant differences were observed in formalin-fixed paraffin-embedded material. These range from a reduction in fluorescence intensity and alteration in binding pattern in the cases of BSA II, Con A, HPA, LCH, PNA and WGA to a complete absence of specific binding in the cases of BSA I, SBA, sWGA and UEA I. These results are in agreement with the recent realization that carbohydrate components are affected by formalin fixation (Brandtzaeg, 1982) and with the report by Ritman & Mackenzie (1983) that aldehyde fixatives proved unsuitable with all lectins in mouse epidermis. Also as routine fixation is a relatively unquantified procedure the observed increase in sample to sample variation is to be expected. As a consequence of the chemical modifications during processing the glycoconjugates shown by lectins in paraffin sections are one stage further removed from the situation *in vivo* than are frozen sections. This problem will be further exacerbated by treatments designed to unmask binding sites (e.g., exposure to proteolytic enzymes) as there is no guarantee that the exposed sites will be the same as those found *in situ* (Finley & Petrusz, 1982). The use of paraffin sections cannot therefore be recommended for investigations of cellular glycoconjugate biochemistry, nor can they be compared directly with frozen sections. However, they may be of use to screen pathological tissue for differences which may be of use diagnostically and as a preliminary to more detailed analyses of unfixed material.

Thirdly, in the case of BSA I and UEA I, previously unreported individual variation in reaction intensity was detected. Though further work is required to establish the molecular basis of this variation, e.g. examination of blood group and secretor status, such variation is to be expected by analogy with other cell types. Inter-species differences in lectin binding have already been noted in mouse (Nemanic *et al.*, 1983) and bovine epidermis (Bell & Skerrow, unpublished observations) and other tissues (Holthoffer, 1983). In practical terms the occurrence of individual variation dictates that sufficient samples should be examined and that in any study of abnormal epidermis an uninvolved control should be obtained from the same individual. Also as minor differences were noted with body site and as regional variations in epidermal glycoproteins have been reported (Goldberg & Bystryn, 1983), this control sample should ideally be from a similar body site.

In conclusion the results of this study suggest that the current discrepancies in the literature may be ascribed to variation between lectin preparations (especially Con A, LCH, UEA I, BSA I), tissue-processing methods (especially BSA I, SBA, UEA I, sWGA), the use of weak saccharide inhibitors (WGA, sWGA) and individual variation within small sample numbers (BSA I, UEA I).

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Epidermolysis bullosa: preliminary observations of blister formation in keratinocyte cultures

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SUMMARY

Keratinocytes from patients with different types of epidermolysis bullosa (EB) demonstrated an abnormal tendency to blister or bleb formation after 14–22 days in culture. The changes were most marked in samples from cases of junctional EB and were not observed in control cultures of normal skin. These findings suggest the possibility of a primary abnormality of keratinocyte adhesion in different varieties of EB and that keratinocyte culture should provide a useful model for further studies on disorders of adhesion in EB.

In the three major forms of epidermolysis bullosa (EB) dermo-epidermal separation occurs at different ultrastructural levels, and is associated, in certain variants, with specific structural or biochemical abnormalities (Eady & Tidman, 1983). In EB simplex, blister formation occurs above the dermo-epidermal junction (DEJ) as a result of keratinocyte cytolysis (Pearson & Spargo, 1961), the mechanism for which is unknown. In the dystrophic forms of EB, separation occurs in the superficial dermis, beneath the lamina densa of the DEJ, and appears to be associated with a numerical reduction of anchoring fibrils (Briggaman & Wheeler, 1975; Hashimoto *et al.*, 1976c) and, in the recessive forms, with abnormal collagenase production (Bauer, 1982). In junctional EB, cleavage occurs in the lamina lucida of the DEJ, and in the more severe form of this condition the sub-basal dense plates of the hemidesmosomes are defective (Hashimoto *et al.*, 1976a).

Certain basement membrane-associated macromolecules including laminin and bullous pemphigoid antigen, which are localized to the lamina lucida (Foidart *et al.*, 1980; Shaumberg-Lever *et al.*, 1975; Holubar *et al.*, 1975), are thought to play a role in dermo-epidermal adhesion.

This work was presented at a meeting of the Investigative Group of the British Association of Dermatologists, Keele University, September 1982.

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It is possible that in junctional EB, in particular, there is an abnormality of one or more of these components of the basement membrane zone.

Laminin and pemphigoid antigen are synthesized by keratinocytes *in vitro* (Woodley *et al.*, 1980; Stanley *et al.*, 1982; Alitalo *et al.*, 1982), so it would be of interest to study the adhesive properties *in vitro* of basal keratinocytes from patients with junctional EB. We have examined various morphological characteristics of cultured keratinocytes from patients with different varieties of EB and report our preliminary observations here.

METHODS

Samples of non-scarred, non-lesional skin were obtained by punch or ellipse biopsy from one adult (aged 59 years) and three children (aged 4 months to 6 years) with junctional EB confirmed by electron microscopy; five patients (aged 3 to 24 years) who, on unequivocal clinical and ultrastructural criteria, suffered with mutilating recessive dystrophic EB; and one child (aged 1 year) with generalized EB simplex confirmed by light microscopy. The junctional EB group was heterogeneous. The three children were severely affected and possessed structurally abnormal hemidesmosomes whereas the adult patient had clinical features of the non-lethal form of this condition (Hashimoto, Schnyder & Anton-Lamprecht, 1976b; Hintner & Wolff, 1982) with structurally normal hemidesmosomes (Tidman & Eady, unpublished). Control keratinocytes were obtained from three neonatal foreskin and three adult surgical skin specimens.

The skin samples were trypsinized (Rheinwald & Green, 1975) and seeded at a very low density (10^3/ml) onto collagen-coated dishes in the presence of 3T3 feeder cells using RPMI 1640 culture medium supplemented with 10% fetal calf serum, cholera toxin (50 ng/ml) and hydrocortisone (5 μg/ml). The same batch of fetal calf serum and the same culture conditions were used throughout. 3T3 feeder cells were used to enable clonal growth and to optimize primary adhesion by the production of basement membrane macromolecules.

Cultures were examined and photographed *in situ* using phase contrast and polarized light microscopy. The keratinocytes were then processed for electron microscopy, both at 14 days before blistering and after blister formation (16–20 days), whilst remaining adherent to the culture dishes, care being taken during fixation and embedding to disturb the cells as little as possible. Primary fixation was for 30–45 minutes in 2% formaldehyde and 2·5% glutaraldehyde, buffered with 0·04 M cacodylate (pH 7·4) containing 5% sucrose and 0·05% calcium chloride. Post-fixation was in 1·3% osmium tetroxide in 0·067 M S-collidine buffer (pH 7·4) for 1 hour on ice. After dehydration in a graded series of ethanol, the cells were embedded in Epon 812 resin. This was achieved by covering the cells with 1:1 Epon–ethanol mixture which was replaced after 30 minutes with neat Epon plus accelerator, and polymerizing at 60°C for 48 hours. Blocks of resin containing the areas of interest in culture, together with the underlying plastic dish, were cut out with a hacksaw and stuck to resin stubs with cyanoacrylate adhesive and orientated for sectioning at right angles to the cell-substrate interface. Semithin (1 μm) sections were stained with Giemsa and examined with a light microscope.

RESULTS

In all samples clonal growth from single cells occurred. In all EB specimens a proportion of the established colonies demonstrated distinctive blistering which was evident macroscopically with phase-contrast microscopy. The centres of the colonies could be seen to have detached from the substrate and to have formed blisters, whilst the cells at the edges remained attached and continued to proliferate towards confluence (Fig. 1). The blisters did not fluctuate in size, and they collapsed when punctured.

Blister formation was most marked in keratinocyte cultures from patients with junctional EB, appearing after 16–18 days in culture, up to 6 days before confluence was reached (Table 1). Sections of the blistered areas confirmed that the lowermost cells had separated cleanly from the substrate (Fig. 2). In one subject (JEB 3), complete separation of the keratinocyte monolayer from the collagen substrate occurred when the cells reached confluence. An identical sequence of events occurred in repeat cultures from further skin samples from this and another junctional EB patient (JEB 1). Cultures which had separated from the substrate failed to passage, presumably because keratinocytes in suspension rapidly undergo terminal differentiation (Green, 1977).

Similar, although less marked, changes occurred in the keratinocyte cultures from the five patients with mutilating recessive dystrophic EB and the one patient with generalized EB simplex. Blister formation occurred rather later (17–21 days) and was not as extensive as in the cultures from junctional EB subjects. Due to technical reasons, it was not possible to section cells from any of the dystrophic EB samples. Primary cultures from the six control specimens did not exhibit blister formation under the same culture conditions, even at confluence and in the presence of 3T3 feeder cells.

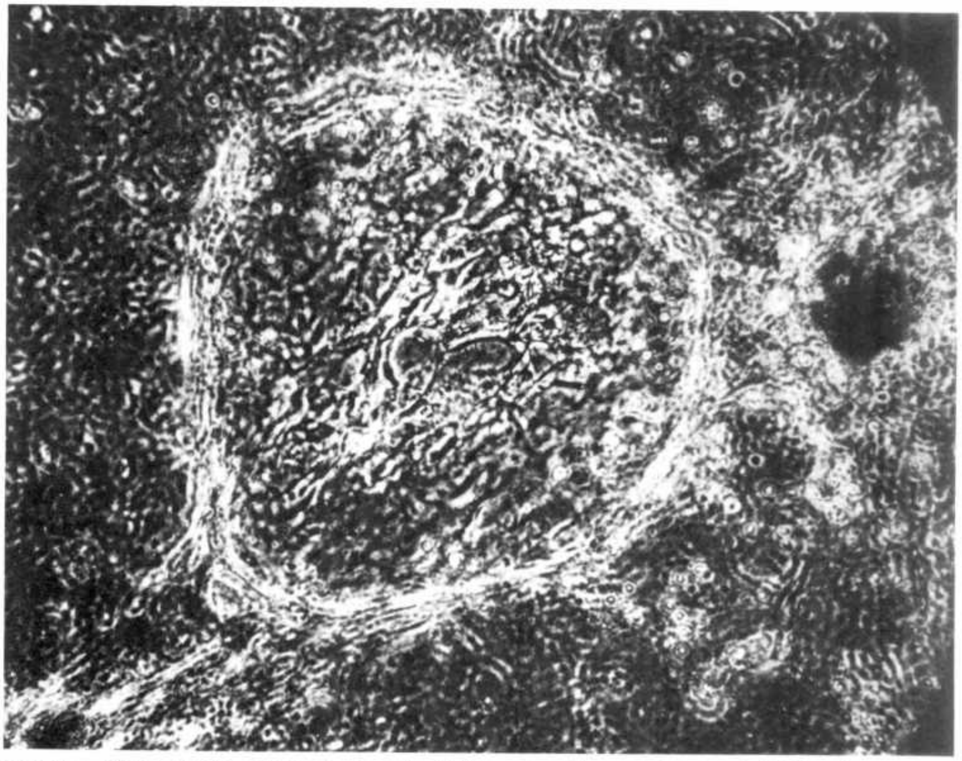

FIGURE 1. Phase contrast photomicrograph of blister in culture of junctional EB keratinocytes (19 days—patient JEB 1).

TABLE I.

Patient	Age Sex	Diagnosis	Age of culture at confluence (days)	Culture morphology Age of culture at blister formation (days)
JEB 1[a]	59 years F	Non-lethal JEB	24	18[a]
JEB 2	4 mo. M	Severe JEB	21	16
JEB 3[a]	1 year F	Severe JEB	21	18[ab]
JEB 4	6 years M	Severe JEB	18	16
DEB 1	3 years M	Mutilating recessive DEB	20	17
DEB 2	13 years F	Mutilating recessive DEB	20	19
DEB 3	24 years F	Mutilating recessive DEB	23	21
DEB 4	22 years M	Mutilating recessive DEB	21	20
DEB 5	3 years M	Mutilating recessive DEB	20	19
EBS 1	1 year F	Generalized EBS	22	20

[a] Repeated with fresh specimens giving identical findings.
[b] Complete separation of monolayer at confluence.
JEB = junctional epidermolysis bullosa.
DEB = dystrophic epidermolysis bullosa.
EBS = epidermolysis bullosa simplex.

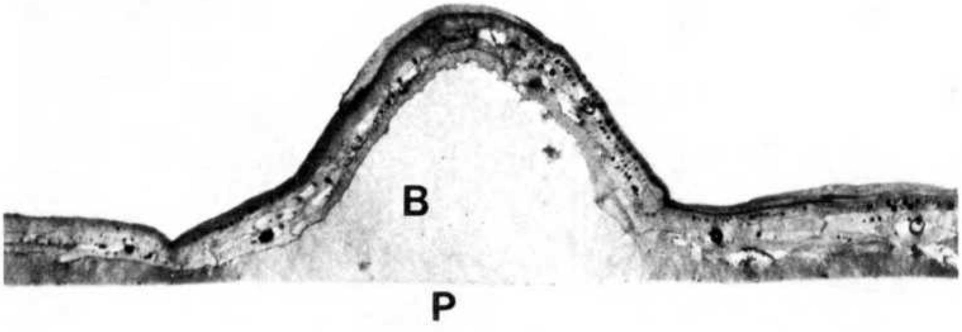

FIGURE 2. Photomicrograph of μm-thick Epon-embedded section of blister (B) in 19-day culture of junctional EB keratinocytes (patient JEB 4). The cells have cleanly detached from the substrate. P = plastic dish. (× 460.)

DISCUSSION

The blisters observed in the present study appear similar to the domes reported to form in confluent cultures of a variety of fluid-transporting epithelia (Leighton *et al.*, 1970; Pickett *et al.*, 1975) and oral epithelium (Birek *et al.*, 1982). However, the term 'domes' has been used to describe collections of fluid within the epithelial layers, sometimes forming pedunculated sacs, and therefore we feel that 'blisters' is a better term to describe our findings in keratinocyte cultures.

The development of domes may be influenced by factors affecting fluid transport (Abaza, Leighton & Schultz, 1974), differentiation (Lever, 1979) or adhesion of cells to the substrate, such as retinoid levels (Adamo *et al.*, 1979). Blistering has previously been noted in cultures of normal keratinocytes grown in delipidized serum, and the tendency was reversed by addition of increased levels of retinyl acetate (Fuchs & Green, 1981).

In our studies the blister formation commenced prior to confluence and with a low cell density, in contrast to doming in other epithelial cultures where a high cell density and confluence are necessary (Young, Cardiff & Seeley, 1978). The marked blistering of keratinocyte cultures from junctional EB patients may reflect the grossly impaired dermo-epidermal adhesion present *in vivo*. Furthermore, the results would suggest that in other forms of EB there is a fundamental abnormality of keratinocyte adhesion. Evidence for such a disorder in mutilating recessive dystrophic EB, in which the primary defect is thought to affect the anchoring fibrils beneath the lamina densa of the dermo-epidermal junction comes from suction blister studies (Tidman & Eady, 1983) that pointed to an abnormality of adhesion within the lamina lucida (i.e., above the lamina densa).

The keratinocyte culture system is a useful model for structural and biochemical studies of the epidermal basement membrane. We suggest that a study of the factors responsible for dome/blister formation in epithelial/keratinocyte cultures may contribute to an understanding of the mechanisms of dermo-epidermal adhesion and the cause of blistering in EB and perhaps in other bullous disorders as well.

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Junctional epidermolysis bullosa in two siblings: clinical observations, collagen studies and electron microscopy

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SUMMARY

Two siblings with junctional epidermolysis bullosa are described: both survived beyond parturition. They were treated with the usual therapeutic doses of phenytoin, dapsone, prednisolone, and zinc supplements without effect. Investigation of the skin of one of the patients showed that his fibroblasts, collagen synthesis and collagenase levels were normal. In view of the normality of the collagenase levels, it is probably not surprising that phenytoin was ineffective. Electron microscopy demonstrated junctional cleavage without pathology in the dermis itself: abnormal hemidesmosomes were seen as described previously, though it is suggested that this is not the primary abnormality which results in the disease process.

Junctional epidermolysis bullosa (JEB) is an extremely rare bullous disorder of the skin which was first described by Herlitz (1935). The disorder is transmitted by an autosomal recessive gene which causes blistering of the skin at the dermo-epidermal junction resulting from trauma. Gedde-Dahl's (1981) classification of epidermolysis bullosa (EB) recognized five variants of the junctional disease, whilst Eady & Tidman (1983) reduced this to three: (a) the lethal form (Herlitz), (b) the non-lethal generalized and (c) the non-lethal inverse form, in which the hands, feet, elbows and knees are relatively spared.

JEB is very often fatal, with most patients dying at or soon after birth. Clinically there is extensive blister formation precipitated by minor trauma, which generally heals without scarring. In addition there is often an accompanying iron deficiency anaemia which is characteristically refractory to treatment. We report studies on two patients with JEB, a brother and sister, the third and fourth children, respectively, of parents who are first cousins.

PATIENTS

Case 1 was a Saudi Arabian boy, aged 2 years 3 months, having been born in Riyadh, weighing

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3 kg, following a normal pregnancy. At birth, blisters were noted on the hands, which rapidly spread in the early days of life to become widespread. The blisters were precipitated by minor trauma during general caring procedures: they contained clear fluid and healed without scarring, leaving hypopigmented areas.

At the age of 1 year he developed facial ulceration which did not heal and spread to involve the anterior nares eventually producing obstruction due to scarring (Fig. 1).

On admission to hospital, he showed evidence of failure to thrive, with height and weight both below the 3rd percentile. There were extensive bullae with eroded areas generally distributed over the body, including the face where there was marked ulceration of the upper lip and scarring of the nose around the nostrils. The teeth were grossly carious and the nails markedly dystrophic (Fig. 2). In addition there was a left corneal opacity.

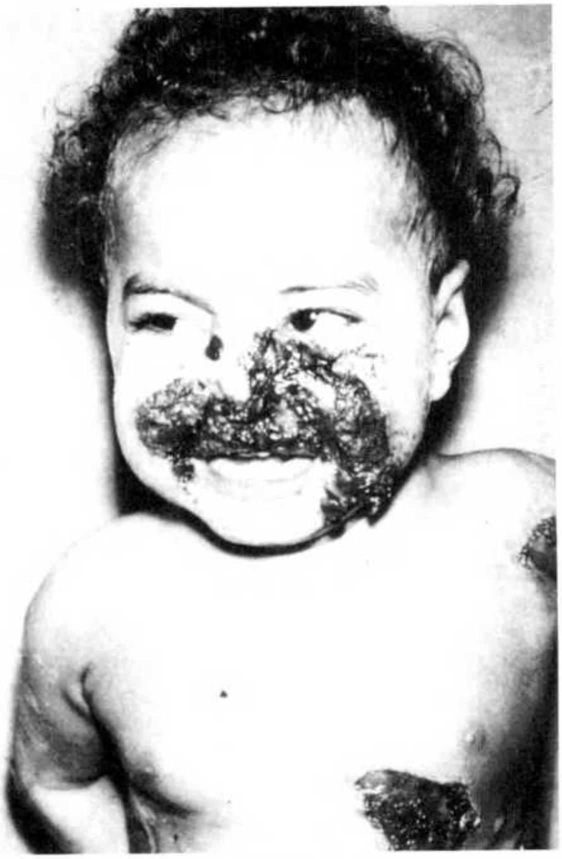

FIGURE 1. Patient 1. Face and upper trunk, showing ulceration of upper lip and left cheek, with destruction of nose due to scarring. Erosions resulting from ruptured bullae are seen on the trunk.

On investigation he had iron deficiency anaemia. The serum zinc level was also low at 4 μmol/l (normal range: 10–22 μmol/l).

Stool examination revealed cysts of *Giardia lamblia* on microscopy. Bacterial culture of the skin lesions grew *Staphylococcus aureus* in moderate numbers.

Initial treatment consisted of occlusive dressings of the larger raw areas with topical

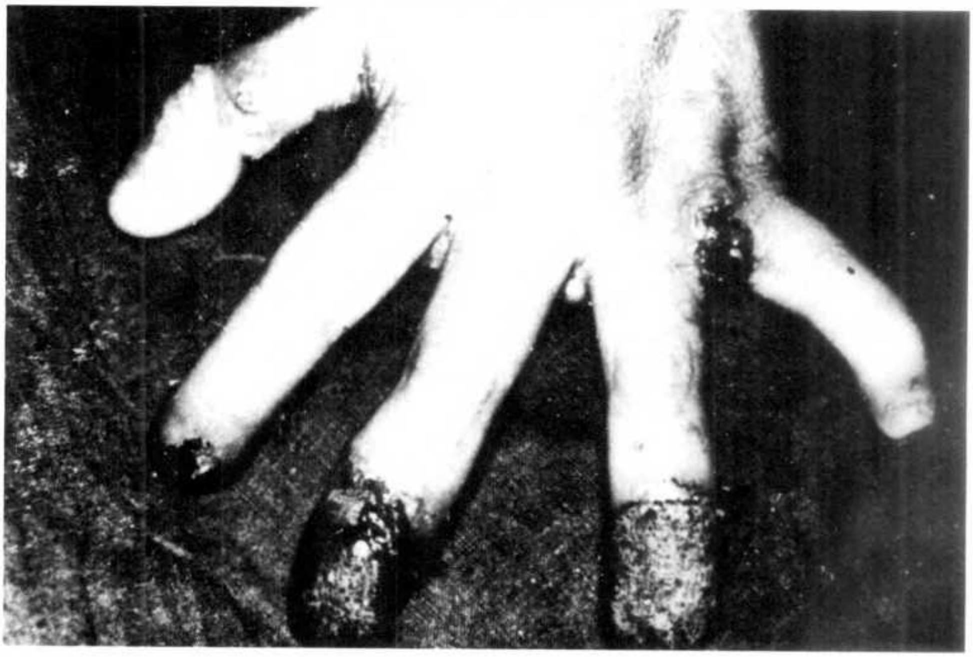

FIGURE 2. Patient 1. Destruction of the finger nails and haemorrhage of the nail bed.

soframycin (Sofra-tulle®), fusidic acid ointment and oral flucloxacillin. The *Giardia* cysts were eradicated with a 5-day course of metronidazole. In view of the anaemia and zinc deficiency, oral iron and zinc supplements were also administered. Phenytoin was given in doses sufficient to produce serum levels of 40–80 μmol/l, and over a period of 1 month no therapeutic benefit was observed. Treatment was then changed to dapsone, which after 2 weeks produced no response and, because of an induced haemolytic anaemia, was discontinued. Finally, oral prednisolone at a total daily dose of 20 mg was administered for a period of 3 weeks, and on discharge from hospital no appreciable improvement had taken place. In addition the anaemia had not improved, nor had the serum zinc level increased. An additional practical problem was encountered when scarring around the alae nasi blocked the left nostril and occluded the right sufficiently severely to cause obstructive sleep apnoea. This was managed by insertion of an oral airway at night and gentle suction of the nares; latterly he learned to breathe solely by mouth and had had no further episodes of apnoea. He returned to Saudi Arabia taking prednisolone 20 mg daily, and iron and zinc supplements.

Case 2, a Saudi Arabian girl, aged 3½ months, the younger sister of patient 1 by the same parents, was admitted with a history of skin blistering which was noted immediately following birth. This rapidly became generalized (Fig. 3) though the blisters healed well; fortunately her face was relatively spared. She was appropriately edentulous at presentation though she had marked nail dystrophy.

On examination widespread bullae were seen together with marked nail dystrophy; she also had oral candidosis. Investigations once again demonstrated an iron deficiency anaemia, and low serum zinc levels (9 μmol/l).

Her management was similar to that of her brother and consisted of successive treatment with phenytoin, dapsone and prednisolone. Staphylococcal infection of the skin was treated with

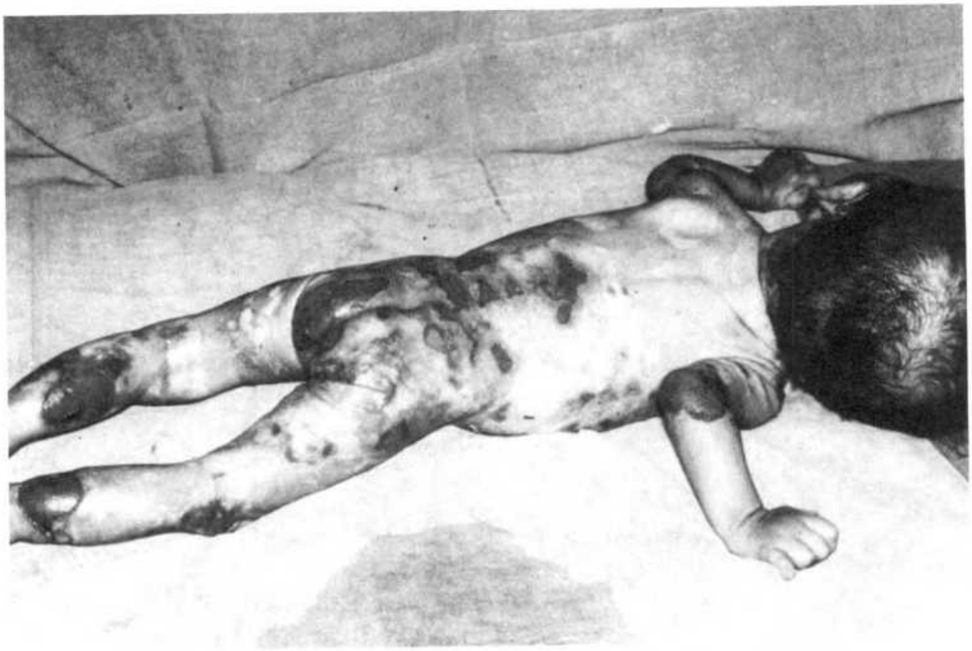

FIGURE 3. Patient 2. Erosions on trunk and limbs due to ruptured bullae.

topical fusidic acid ointment and oral flucloxacillin. Neither phenytoin nor dapsone produced a beneficial response, though systemic steroids appeared to reduce the frequency of blister formation slightly. She returned to Saudi Arabia taking prednisolone 10 mg daily and iron and zinc supplements.

METHODS

The tissue was obtained from an incisional biopsy of the skin of the chest and was fixed in 3% glutaraldehyde in 0·1 M sodium cacodylate buffer (pH 7·4), post-fixed in 2% aqueous osmium, dehydrated and embedded in Spurr resin. Thin sections were stained with uranyl acetate and lead citrate before examination in a Philips 301 electron microscope.

Fibroblast cultures were established from an incisional biopsy of the skin of the chest as previously described (Priestley & Adams, 1983).

Proliferation rates were determined for fibroblast lines as previously described (Priestley & Adams, 1983).

The method used to study collagen synthesis has previously been described (Priestley, 1983) with the exception that [^{3}H]proline was added in culture medium containing 2% fetal calf serum.

To study collagenase activity, 25 mCi of [^{3}H]acetic anhydride (Amersham International, U.K.) was used to label acid-soluble calf skin collagen (Sigma Chemical Co., Poole, U.K.), following the method of Lefevere, Slegers & Claeys (1979), and this acted as the radio-labelled substrate in the assay. To estimate collagenase activity, medium from three confluent flasks (175 cm^2 growth area) of a fibroblast cell line was removed and the cells were washed five times with phosphate-buffered saline. Twenty millilitres of serum-free medium was added to each flask before incubation at 37°C for 72 h. The medium was then pooled, and the cells were resuspended in trypsin–versene and the total cell number was estimated by counting aliquots of cells in a Coulter Counter. Pooled medium was concentrated tenfold to a final volume of 6 ml by vacuum dialysis at 4°C through Millipore immersible-CX ultrafilters (nominal pore size 10,000 daltons). The concentrate was dialysed for 24 h at 4°C against 0·05 M Tris–HCl buffer (pH 7·5) containing 5 mM $CaCl_2$. Collagenase activity was then measured following trypsin activation as previously described (Bauer, Stricklin & Jeffrey, 1975). 100-μl aliquots of the final supernatant were diluted in 1 ml water and added to 10 ml of scintillant composed of Triton X-100 and NE233 (Nuclear Enterprises, Edinburgh, U.K.), in a 1:2 ratio. Radioactivity released from the labelled substrate by the collagenase in the medium preparation, was then measured using a Packard Tri-carb 300 liquid scintillation counter.

RESULTS

Electron microscopy

Cleavage of epidermis from dermis, which occurred spontaneously during the biopsy procedure (Fig. 4), took place between the basal cell plasma membrane and the basal lamina. This has previously been shown by Pearson (1962, 1967). Where the plasma membrane remained intact, hemidesmosomes could be seen with anchoring filaments still attached. Perinuclear oedema and numerous vacuoles were striking features of the basal cells which formed the blister roof (Fig. 5). The desmosomes were normal in appearance but the associated tonofilaments, though present, had been disrupted by the large vacuoles within the basal keratinocytes.

The somewhat diffuse basal lamina could be seen in the floor of the blister (Fig. 6). Associated with it was some amorphous material and anchoring filaments as well as more densely staining fragments of basal cell cytoplasm attached via hemidesmosomes. Although these hemidesmo-

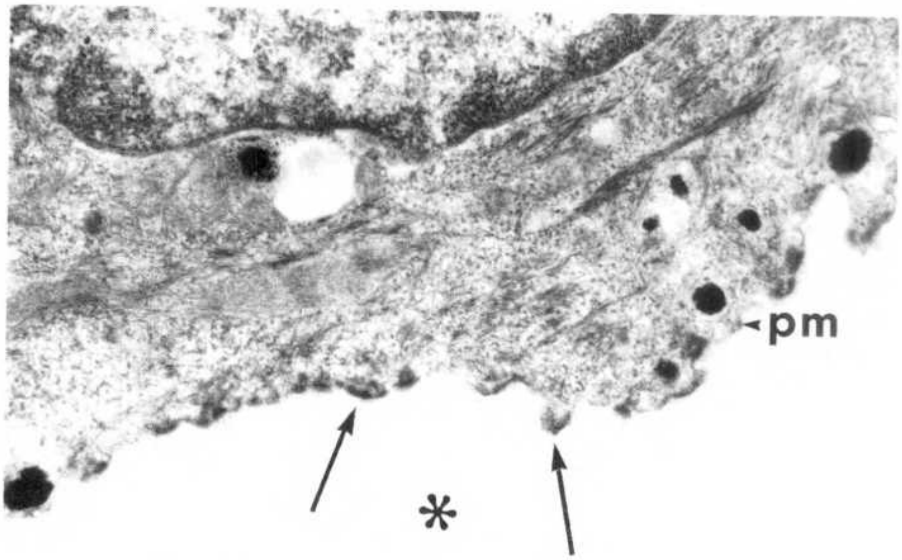

FIGURE 4. Blister roof. The plasma membrane (pm) of the basal cells has remained intact. Anchoring filaments (arrows) remain attached to the hemidesmosomal plaques. The blister cavity is marked with an asterisk (*). (× 25,500.)

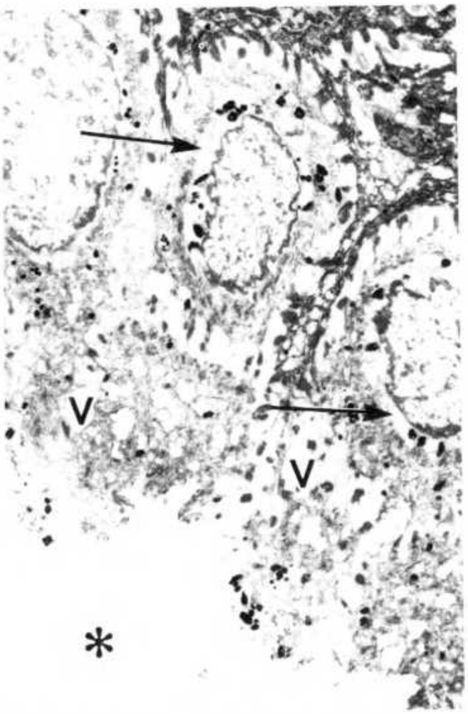

FIGURE 5. Blister roof. The basal cells overlying the blister cavity (*), show perinuclear oedema (arrows) and are highly vacuolated (V). (× 4400.)

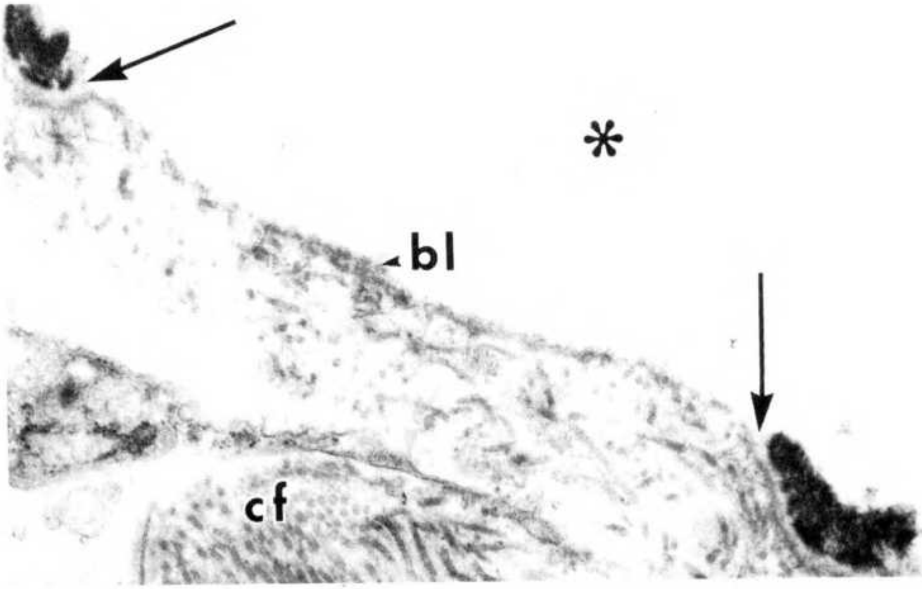

FIGURE 6. Blister floor. The basal lamina (BL) overlies the dermis, where normal collagen fibres (cf) are present. Portions of basal cell plasma membrane and cytoplasm have remained attached via hemidesmosomes (arrows) to the basal lamina during cleavage. The blister cavity is marked with an asterisk (*). (× 22,800.)

somes were characteristically electron-dense, with anchoring filaments attaching them to the basal lamina, there appeared to be no sub-basal plaque present. Anchoring fibrils could be seen on the dermal aspect of the basal lamina, extending into an apparently normal dermis. The collagen of the dermis appeared normal with no evidence of degenerative changes.

In areas where the basement membrane zone had remained intact the junction appeared to be normal (Fig. 7). Hemidesmosomes could be seen though they varied in the amount of electron-dense material which formed the plasma membrane attachment plaque and lacked the electron-dense plaque in the sub-basal area. Between hemidesmosomes a number of microcytotic vesicles could be seen associated with the plasma membrane of the basal keratinocytes. The basal lamina was not of a uniform density and there was evidence of disruption with duplication occurring in places. Normal anchoring fibrils could be seen attached to the basal lamina. The underlying dermis appeared normal.

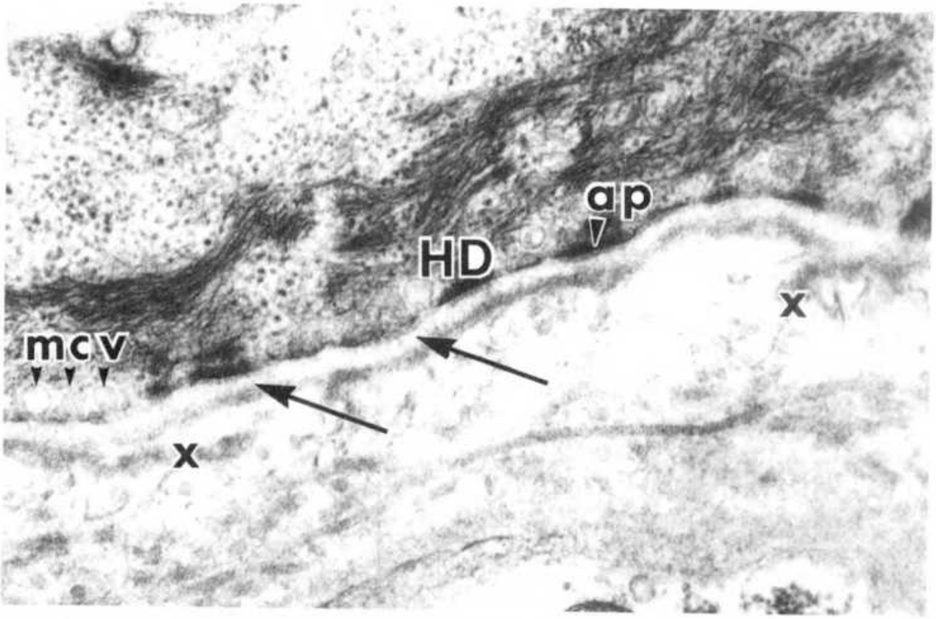

FIGURE 7. Intact skin. At the dermo-epidermal junction hemidesmosomes (HD) with variable amounts of material forming their plasma membrane attachment plaques (ap), can be seen. Anchoring filaments (arrow) traverse the lamina lucida. Microcytotic vesicles (mcv) are present above the basal cell plasma membrane and disruption of the basal lamina is evident (X). (× 47,500.)

Investigations of JEB fibroblasts

Results are from a single JEB cell line (Patient 1) and therefore give only an indication of JEB fibroblast characteristics. The results are summarized in Table 1.

No statistical significance can be attached to the data as a group is being compared with a single sample. However, rates of proliferation and collagen synthesis, both in relative and absolute terms, fall within the normal control range. Non-collagen protein synthesis was at the upper end of the normal range (11,998–60,738 dpm) but as the collagen synthesis per cell is also

TABLE 1. Collagen synthesis as a proportion of total protein synthesis

	Control ($n = 8$) (mean ± S.E.M.)	JEB ($n = 1$)
Proliferation rate (%)	99 ± 13	139
Relative collagen synthesis (%)	3·2 ± 0·5	3·2
Collagen synthesis/10^5 cells (dpm)	5425 ± 1608	8,364
Non-collagen synthesis/10^5 cells (dpm)	29,198 ± 5,842	51,164

Control lines were derived from forearm skin biopsies of five males aged between 5 and 37 years and three females aged between 22 and 25 years. The JEB fibroblast line was derived from a chest skin biopsy from the 2-year-old boy (case 1); five separate collagen assays were performed on the cell line, confirming reproducibility.

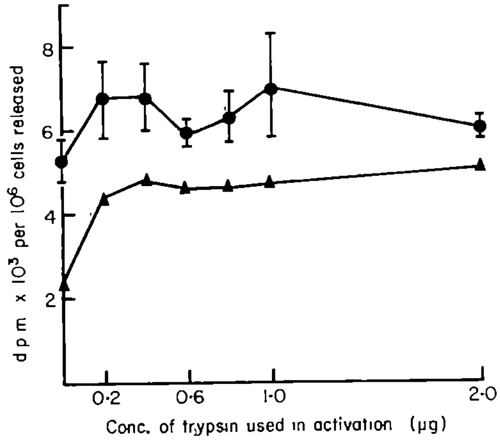

FIGURE 8. Collagenase activity in fibroblast cultures from the skin of patient 1 with JEB, and four controls. The radioactivity released following trypsin activation is shown at different concentrations of the trypsin. In the control group, the mean and s e.m. are indicated.

TABLE 2. Initial and mean activated levels of collagenase activity in JEB and control groups

	Control (mean ± S.E.M.)	JEB
Initial collagenase level	5286 ± 300	2349
Activated collagenase level (d.p.m./10^6 cells)	6931 ± 246	4748

To obtain overall levels of collagenase activity in the control lines described in Table 1 before and after activation, the results for final levels of collagenase activity have been pooled.

well above the mean, this probably indicates a general increase in cellular function over the average normal control, possibly due to the age of the donor.

The collagenase results are shown in Fig. 8. Again there is no significant difference between JEB and the control group, the JEB-activated collagenase levels being slightly lower than the average control values (Table 2).

DISCUSSION

Pearson (1962, 1967) was among the first to establish, by electron microscopy techniques, that in JEB, blister formation occurred between the basal cell plasma membrane and basal lamina. More recently Hashimoto *et al.* (1976) and Anton-Lamprecht & Schnuder (1979) concluded, from detailed EM studies, that an abnormality of the hemidesmosomes was the primary causal defect in this variety of EM. Our observations show the hemidesmosomes to be abnormal, but whether this is the primary defect remains unanswered.

Anchoring filaments are present beneath the hemidesmosomes where the electron-dense sub-basal plaque is absent or more diffuse. This agrees with the findings of Rodeck, Eady & Gosden (1980). Absence of this plaque could represent the weak link in the attachment of epidermis to dermis although there is evidence against this: firstly, electron microscopy (Fig. 4) shows that anchoring filaments do remain associated with the hemidesmosomes after cleavage; Pearson, Potter & Strauss (1974) also noted the retention of material beneath hemidesmosomes in cleaved skin. Secondly, the 'torn off' phenomenon (Hashimoto *et al.*, 1976) (Fig. 6) suggests that the hemidesmosomes can maintain their connection with the basal lamina during the cleavage, resulting in rupture of the basal cell plasma membrane. In addition morphometric analysis by Tidman & Eady (1983) has shown that junctional EB is an ultrastructurally heterogeneous condition, three out of seven patients having structurally and numerically normal hemidesmosomes despite marked reduction in dermo-epidermal adhesion. 'Hypoplastic' hemidesmosomes as reported by Hashimoto *et al.* (1976) and Anton-Lamprecht & Schnuder (1979) cannot therefore be assumed to be the primary causal defect.

The significance of the microcytotic vesicles is uncertain. They are related to the lysosomal system, possibly having the potential to release lysosomal enzymes capable of degrading collagen, proteoglycans and protein (Lazarus, Hatcher & Levine, 1975). Undoubtedly the basal cells do produce enzymes capable of causing detachment from the basal lamina during normal division and differentiation. Our findings, including the numerous vacuoles disrupting the basal keratinocytes reported here (Fig. 5) and by Pearson (1962), suggest that an abnormality within the basal cells could be responsible for the lack of stability at the dermo-epidermal junction which is characteristic of JEB. Alternatively, these vacuoles may be a secondary change following cleavage of the dermo-epidermal junction.

The evidence for collagenase involvement in the pathogenesis of JEB is tenuous. Evidence from this study agrees with the findings of Bauer & Eisen (1978), who found normal collagenase levels in skin fibroblasts from JEB patients. Although in contrast to the *in vivo* findings (Bauer, Gedde-Dahl & Eisen, 1977), it is now thought that the increase in collagenase was a secondary response to chronic wound healing (Bauer & Eisen, 1978). Abnormalities of collagenase metabolism have only been associated with dystrophic recessive EB (EBDr) (Bauer, 1982). Phenytoin is recognized as being useful in treating some EBDr patients (Bauer *et al.*, 1980) through its indirect effect on synthesis and/or secretion of collagenase; as there is no indication of abnormal collagenase metabolism in JEB, there are no grounds for its use in this disorder and this is borne out in the two patients described. Furthermore, there is evidence that the use of phenytoin in JEB may be detrimental to the course of the disease (Bergfeld & Orlowski, 1982).

In the patients described dapsone was tried, as it is known to be of benefit in some other bullous disorders, but without effect. Steroids were also used: Pearson *et al.* (1974) used prednisolone in three patients with junctional EB with some apparent improvement, whilst Schachner, Lazarus & Dembitzer (1977) found a lack of response in his patients to corticosteroids. Interestingly, one of Schachner's patients with non-lethal junctional EB responded to hormone treatment (Enovid E®), after pregnancy had precipitated the disappearance of all blistering.

In common with many of the reported JEB cases, our patients had iron deficient anaemia (Hruby & Esterly, 1973; Pearson *et al.*, 1974). Pearson concluded that anaemia in longer surviving patients could not be explained by iron deficiency alone or blood loss but appeared to be due to bone marrow dysfunction which he presumed to be secondary to the disease itself. Our patients also had zinc deficiency which could be important in preventing healing (Husain, 1969; Serjeant, Galloway & Gueri, 1970; Halbrook & Lanner, 1972). Whether this was nutritional or secondary to the disease is not known: it seems that the latter possibility is the more likely, as the serum zinc levels did not rise with zinc supplements.

In conclusion, our attempts to ameliorate the childrens' JEB by using drug therapy have proved ineffectual. At present only careful aseptic nursing seems to be of benefit; it is likely that this will continue to be the case until further inroads into our understanding of JEB have been made.

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Dermographism (mechanical urticaria) mediated by IgM

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SUMMARY

Sera of three patients with symptomatic mechanical urticaria caused an immediate weal and flare reaction when injected intracutaneously into related recipients, and serum of one patient caused the same reaction in his own skin. Pretreatment of test skin sites with Compound 48/80 abolished or greatly reduced the reaction. This weal-producing substance was found in the same fraction as IgM in Sephadex G-200 gel filtration, was stable to heat treatment (56°C, 4 h) and labile to 2-mercaptoethanol treatment. An immunoadsorption study using anti-IgM serum identified this weal-producing substance as IgM.

Mechanical urticaria (dermographism) is the name given to urticaria inducible by firm stroking of the skin with a hard material such as a nail or a sharp rod. Its pathogenesis is largely unknown, but successful passive transfer of dermographism has been described in which the transfer substance was either IgA or IgE (Aoyama, Katsumata & Ozawa, 1970; Newcomb & Nelson, 1973; Jorizzo & Smith, 1982; Goevic & Kaplan, 1980). It is therefore widely accepted that in mechanical urticaria, mast cells sensitized with immunoglobulins (particularly IgE) are activated in response to mechanical stimulation of the skin, although the nature of the reacting antigens is not clear. This paper describes the detection of weal-producing IgM in the sera of dermographic patients. This may present another possible mechanism for mechanical urticaria.

METHODS

Patients

The three patients studied all produced a weal and flare accompanied by itching only in response to mechanical stimulation.

Case 1. A 15-year-old girl had suffered from symptomatic dermographism for 16 months. Otherwise she had been well. No family or personal history of atopy was noted. Contact of the skin with warm water (40°C), running water (18°C) or an ice cube for 5 min respectively did not cause any abnormal reaction, but scratching of the skin with the back of a pencil produced a marked linear weal. Intracutaneous skin tests with ordinary food and inhalant allergens were positive only to house dust. Routine laboratory examinations including complete blood count, urinalysis, liver function tests, serum protein electrophoresis and serum immunoglobulin levels were normal. Rapid plasma reagin (RPR) test for syphilis and serum hepatitis B surface (HBs) antigen were negative.

Case 2. A 21-year-old man gave an 18-month history of linear weal formation accompanied by slight itch whenever his skin was rubbed with a hard substance. He had been otherwise healthy. There was no family or personal history for atopy. On examination there was no spontaneous wealing nor did he produce weals in contact with warm water, running water or an ice cube. He produced weals only in response to mechanical stimulation. Complete blood counts, urinalysis, liver function test, serum protein electrophoresis and serum IgE levels were normal. Serum RPR test and HBs antigen were negative.

Case 3. A 16-year-old boy gave a 3-month history of symptomatic dermographism. Family and personal history for atopy were negative. The ice cube test was negative, and contact with warm water and running water did not cause wealing. Skin tests with food and inhalant allergens revealed positive immediate reactions to house dust and candida. Laboratory examinations including complete blood count, urinalysis, liver function test, serum protein electrophoresis and serum immunoglobulin levels were normal. Serum anti-streptolysin O, C-reactive protein, rheumatoid factor, RPR test and HBs antigen were negative.

Skin testing

All skin test materials were sterilized using Milex GS filter (Millipore, 0·22 μm pore size) and 0·1 ml of these was injected intracutaneously in the ventral aspects of the forearm or in the back. Weal and flare reactions were read at 15 min and their relative intensities were compared.

Passive transfer test

The passive transfer test was performed only when the following conditions were fulfilled.

(1) The patient had no experience of blood transfusion and had no history of hepatitis or jaundice.

(2) The patient was negative for serum HBs antigen by reversed passive haemagglutination test and had normal liver function tests.

(3) The patient and the recipient were very keen to know the cause of the disease.

(4) Both the patient and the recipient understood the purpose of the study and accompanying risks, and agreed to the test.

The recipient was in every case a family member living with the patient and eating the same food. Thus the recipients were the fathers of cases 1 and 3 and the mother of case 2. After phlelotomy the blood was spun at 3000 r.p.m. for 10 min and the serum was obtained. This was diluted 1:2 to 1:32 and further if necessary with saline, and 0·1 ml each was skin-tested in the forearm of the recipient.

Mast cell exhaustion

Several sites in the ventral skin of the recipient were injected with 0·1 ml each of 0·02% Compound 48/80 in saline.

Gel filtration

This was performed using a Sephadex G-200 column (2·5 × 105 cm) equilibrated with physiological saline. The patient's serum (1·5 ml) was applied and eluted to obtain 5 ml fractions with physiological saline instead of buffer solution (to avoid pain on injecting effluent). Optical density was read at 254 nm. Every two fractions (0·1 ml each) were skin tested in the back of the recipient.

Distribution of IgG, IgA and IgM in the fractions was determined by the semi-quantitative immunodiffusion method (Parker, Aoki & Turk, 1970) using commercially available monospecific antiserum against human IgG, IgA and IgM (Hoechst). IgE concentrations of the fractions were determined by radioimmunosorbent test using a commercial kit (Pharmacia).

Heat treatment

An aliquot of the patient's serum was incubated in a water bath at 56°C for 2 h and cooled, and 0·1 ml each of this and untreated serum were skin-tested in the forearm of the recipient.

2-Mercaptoethanol (2-ME) treatment

The patient's serum (0·1 ml) was mixed with the same volume of 0·1 M 2-ME and left for 15 min at room temperature. The mixture was then mixed with 0·2 ml of 0·1 M idoacetamide (final dilution of the serum four times), and after 1 h at room temperature this was dialysed for 24 h against three changes of saline solution (1 litre each). The dialysed solution and the untreated serum diluted four times (0·1 ml each) were skin-tested in the forearm of the recipient.

Immunoadsorption

Monospecific antiserum against human IgG and IgM was produced in our laboratory by immunizing rabbits with purified myeloma IgG or IgM and absorbing unnecessary antibodies contained in the antiserum with newborn cord serum or human serum unprecipitable with 35% ammonium sulphate. Monospecificity of the serum was finally confirmed by immunoelectrophoresis against whole human serum (Aoki, Parker & Turk, 1969). Gammaglobulin fractions precipitated with 35% ammonium sulphate from the monospecific anti-IgM and anti-IgG antiserum and normal rabbit serum were insolubilized as described by Avrameas & Ternynck (1969) using glutaraldehyde. For immunoadosorption, washed insolubilized antiserum (0·8–1·0 ml packed volume) was mixed with 0·2 ml of patient's serum and incubated at 37°C for 60 min with repeated agitation by a mixer and left overnight at 4°C. The mixture was centrifuged at 1500 r.p.m. for 5 min and the supernatant was analysed for its immunoglobulin content, using LC-Partigen-IgM and Tri-Partigen-IgG (Hoecst) and was skin-tested in the forearm of the recipient.

RESULTS

Passive transfer (P-T) test

In all three cases the patient's serum caused a weal and flare reaction in the skin of the recipient that was maximum at 15 min and disappeared at 30 min. This reaction occurred spontaneously without giving any stimulus such as stroking on the test site. The maximum dilution to give a positive reaction was 1 : 128 in two cases and 1 : 8 in the other. Stroking on the test sites 24 h later did not cause any reaction. As controls, three normal human sera were skin-tested in the same way in three normal subjects (including the authors) but a weal and flare reaction was not observed in any subjects. The patient's serum was later tested in the patient's own skin in cases 1 and 3, and a weal and flare reaction similar to that observed in the recipient was noted in case 1 only.

Gel filtration

This was performed in all three cases and the results were the same, so details are given only for case 1. As shown in Fig. 1, weal-producing activity was detected only in the first peak, which just coincided with the IgM distribution.

Effect of heat and 2-ME treatment on P-T test

This was examined in cases 1 and 3. In both cases, the weal-producing activity of patient's serum was not changed by the heat treatment, but was completely abolished by the 2-ME treatment.

Effect of mast cell exhaustion on P-T test

This was studied in cases 1 and 3. When the P-T test was performed 3 h after treatment with Compound 48/80, P-T activity was greatly reduced (Table 1).

Effect of immunoadsorption on P-T test

This was examined in case 1. When the patient's immunoadsorbed serum was skin-tested in the recipient, only the serum adsorbed with anti-IgM (IgM trace, IgG 720 mg/dl) lost its weal-producing activity. The sera adsorbed with anti-IgG (IgM 90 mg/dl, IgG 10 mg/dl) and with normal rabbit serum (IgM 102 mg/dl, IgG 900 mg/dl) showed the same weal-producing activity (Fig. 2).

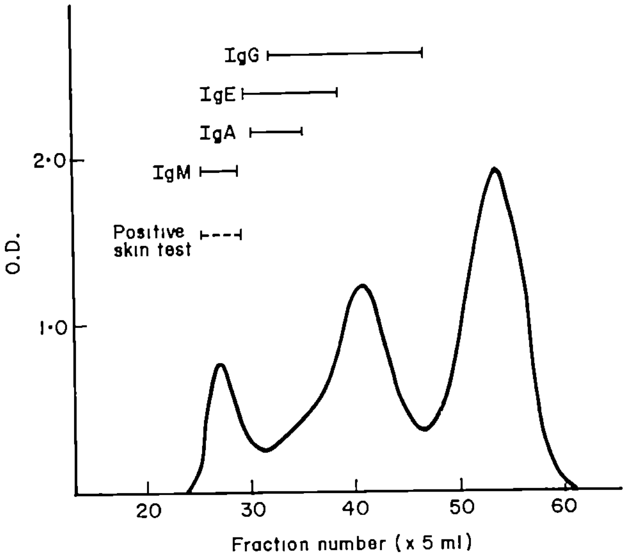

FIGURE 1. Sephadex G-200 gel filtration of the patient's serum and the distribution areas of four immunoglobulins and skin test.

TABLE 1. Effect of mast cell exhaustion on P-T reaction

Case	Treatment with Compound 48/80	Dilution of the serum 1:2	1:4	1:8	1:16	1:32	1:64	1:128
1	Before	++	++	++	+	+	+	+
	After	+	+	+	–	–	–	–
3	Before	+	+	+	–	–	–	–
	After	–	–	–	–	n.d.	n.d.	n.d.

++, + = positive reaction.
– = negative reaction.
n.d. = not done.

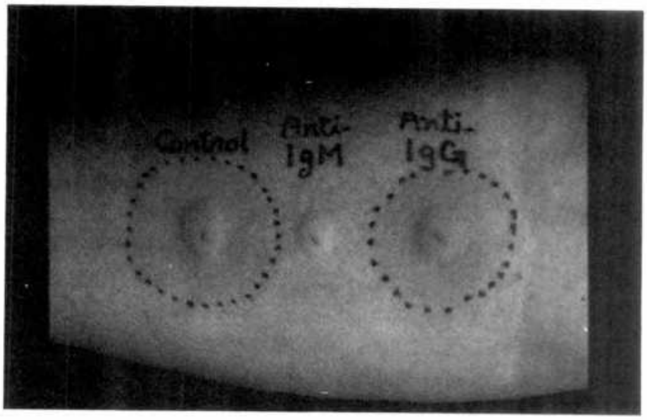

FIGURE 2. Skin tests with the patient's serum immunoadsorbed with normal rabbit serum, anti-human IgM rabbit antiserum or anti-human IgG rabbit antiserum. The dotted lines indicate erythematous areas.

DISCUSSION

Aoyama *et al.* (1970) succeeded in producing passive transfer in nine out of thirteen patients of dermographism, and showed that either IgA or IgE was responsible. Newcomb & Nelson (1973) reported that in five of nine patients passive transfer of dermographism was successful, and in the two patients examined, IgE was confirmed as the mediator. In these cases mechanical stimulation was given to induce weals at sites where the serum of a patient had been injected several hours previously. It is well known that IgE attached to the receptor on the surface of mast cells reacts with antigen leading to degranulation of the cells, but in mechanical urticaria the corresponding antigen has not yet been found. IgM as the cause of urticaria was demonstrated by Wanderer *et al.* (1971) and Inoue *et al.* (1978) in cold-contact urticaria. They showed that a weal and flare reaction occurred when the skin sensitized with the patient's serum was cooled with an ice cube. The intervals between injection of serum and challenge with ice have been between 1·5 and 6 hours, which are shorter than those (several hours to 24 hours) in the reactions mediated by IgE (Houser *et al.*, 1970). Immediate weal and flare reactions mediated by IgM have been described in generalized cold urticaria by Aoki, Horiko & Akimoto

(1982). This was shown to be the result of short skin fixation time, and reactivity at moderate temperature of this IgM. It was suggested that generalized cold urticaria was caused by capillary leakage of this IgM and lowering of skin temperature by sweating.

The results of the present investigation have shown that weal-producing IgM similar to that detected in generalized cold urticaria may also be present in mechanical urticaria. The role of this particular IgM in the pathogenesis of mechanical urticaria is difficult to evaluate. In the usual type of mechanical urticaria, mast cells sensitized with IgE antibody are thought to react with antigens produced in the skin by mechanical stimulation. A similar explanation to that for IgM-transferable cold urticaria is possible, regarding it as a sensitizer of mast cells, although IgM receptor on the surface of mast cells has not yet been demonstrated. The P-T lesion observed in our study cannot be a true model of the spontaneous lesions seen in our patients, since it appeared immediately after introduction of the serum into the skin, and it followed injection of the serum without mechanical stimulation of the test sites. The weal and flare reaction was also observed in one patient when the serum was injected into his own skin. Therefore, other explanations are needed for the IgM we detected, and the following speculations were made. Firstly, IgM may be a substance that makes mast cells fragile, perhaps acting via the supposed IgM receptor, so that stroking the skin can directly cause wealing without the involvement of antigens. Secondly, it may be an antibody to mast cells so it can disrupt the cells after leakage through skin capillaries in the Lewis triple response caused by skin stroking. Further work is required, but our study opens new possibilities in the pathogenesis of mechanical urticaria.

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The role of Substance P in the axon reflex in the rat

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SUMMARY

The increase in vascular permeability in small blood vessels of rat skin induced by antidromic stimulation of sensory roots, nerves, or single identified polymodal nociceptive fibres or by intra-arterial injection of Substance P, has been examined by light and electron microscopy. The effects of local application of capsaicin (a Substance P-depleting agent) to both skin and muscle have also been studied.

Nerve stimulation and Substance P induce leakage of a venular pattern similar to that caused by known permeability factors. However, capsaicin causes leakage from both venules and capillaries in a pattern more characteristic of direct endothelial injury.

It is suggested that neurogenic leakage may be mediated by local liberation of Substance P, but caution is necessary in interpreting experiments involving the use of capsaicin because of its local toxic effects.

For almost a century evidence has accumulated which suggests a role for the nervous system in cutaneous inflammation. Stimulation of dorsal roots evokes cutaneous vasodilatation and plasma extravasation (Bayliss, 1901) if the stimulation is strong enough to excite unmyelinated fibres (Hinsey & Gasser, 1930). This neurogenic effect is independent of the connections between dorsal roots and spinal cord and therefore was ascribed by Bayliss to the 'axon reflex'. Recent experiments have shown that an axon reflex resulting in neurogenic plasma extravasation can only be elicited by stimulation of polymodal nociceptive C fibres (Kenins, 1981). The neurotransmitter for this plasma extravasation is unknown, but considerable evidence supports Lembeck's suggested role for Substance P (Lembeck, 1953). This evidence, as summarized by Lembeck (1983) is that Substance P is found within neurons both in the skin and the spinal cord, if injected it evokes erythema and oedema, and pretreatment with the neurotoxin capsaicin, which depletes Substance P, prevents neurogenic plasma extravasation.

Increased vascular permeability is one of the main features of the acute inflammatory reaction. Two mechanisms may contribute to its production—direct endothelial injury and the

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action on small vessels of chemical substances, called mediators, released within the injured tissues. These two mechanisms may be distinguished by the pattern of vascular labelling seen in animals injected intravenously with colloidal carbon during the period of increased permeability using the criteria established by Cotran & Majno (1964). Leakage induced by chemical mediators is restricted to postcapillary venules, but that due to direct injury occurs from small blood vessels of all types exposed to a supra-threshold injury.

The present experiments were performed to determine the type of increased permeability produced by antidromic nerve stimulation, infusion of Substance P and local application of capsaicin.

METHODS

All experiments were performed on anaesthetized Sprague-Dawley rats, body weight 200–350 g. Urethane (1 g/kg body weight i.p.) was used for all the experiments except those on the cremaster muscle where pentobarbitone (60 mg/kg) was injected into the tail vein. Areas of increased vascular permeability were detected by intravenous injection of Evans' blue (50 mg/kg body weight) 30–60 minutes before killing of the animal. This dye is not trapped by the basement membrane so it more accurately reflects the extent of oedema than the exact area of increased permeability. The pattern of leaking vessels within the area of increased permeability was determined by 'vascular labelling' produced by intravenous injection of colloidal carbon suspension (Gunther-Wagner, Pelikan Werke C11/1431a) in a dosage of 0·1 ml/100 g body weight 1 hour before killing. This colloidal carbon suspension is electron dense and too large to pass through the basement membrane of the blood vessels, so that its entrapment within the vessel walls marks the actual site of increased permeability. By this technique individual carbon-labelled vessels can be identified both in cleared macroscopic specimens and in light and electron microscopic (EM) sections.

In each animal before experimental manipulation began, at least 10 min were allowed to elapse after dye injection to check for existing cutaneous microvascular damage. After the manipulation the animal was kept anaesthetized for 30–60 min to allow all circulating carbon to be removed by the reticulo-endothelial system, then killed by an overdose of the anaesthetic and the appropriate area of skin dissected free, pinned to a piece of cardboard and fixed in 2·5% glutaraldehyde. After fixation for 48–72 hours, cleared skin samples were prepared by dehydration through ascending alcohol concentrations and immersion in cedarwood oil for 2 to 4 days at room temperature. Specimens were viewed through a dissecting microscope under epi-illumination.

EM sections were prepared by cutting 3 × 1 mm blocks from labelled areas of glutaraldehyde fixed specimens, post-fixing these in 2% osmium tetroxide and embedding them in Spurr's resin. Thick (0·5 μm) sections were cut, stained with methylene blue in borax and viewed by light microscopy. Areas of interest were identified and thin sections of these areas cut with a diamond knife, stained with lead acetate and uranium nitrate and examined in a Siemens 102 electron microscope at 60 kV.

To determine the pattern of leakage caused by antidromic stimulation of the cut dorsal roots (DR) or of the cut saphenous nerve, six rats were anaesthetized with urethane, their hind quarters shaved and tracheal and jugular vein cannulae were inserted. Either a laminectomy was performed to expose the DRs, L3,4,5 supplying the hind leg, or the saphenous nerve was freed in the upper thigh and cut centrally. Carbon suspension and Evans' blue were injected intravenously to check for existing damage, then the distal section of either a dorsal root or the saphenous nerve was placed on hook wire electrodes and electrically stimulated (15 V × 10 Hz × 0·5 msec duration) for 5 min. The animal was killed 1 hour later and specimens prepared as described above.

RESULTS

Effects of electrical stimulation of dorsal root or peripheral end of severed saphenous nerve

Both these types of stimuli produced an area of cutaneous blueing confined to the innervation territory of the stimulated nerve. Within this area scattered carbon-labelled venules were visible and this labelling was restricted to venules, but not all vessels of this type within the blued area were labelled.

As shown in Fig. 1A the resulting pattern of leakage was of the mediator type and this was checked by EM (Figs 1B and 1C) for the increased permeability from the antidromic stimulation of a single polymodal nociceptive C fibre carried out as previously described (Kenins, 1981). Within the area of blueing created by the Evans' blue dye leakage only isolated areas of carbon deposit could be found. Due to the sparseness of carbon leakage it was not possible to determine fully the extent of congruence of Evans' blue and carbon deposition without ultrathin serial sectioning and EM of the whole skin sample. However, it appeared that the area of Evans' blue-stained tissue exceeded the area of carbon-impregnated tissue, and only some of the blood vessels within the area exhibited leakage. As shown in Figs 1B and 1C, carbon particles (arrows) were found only within the basement membrane of post-capillary venules confirming a mediator type of response.

Effects of infusion retrogradely of Substance P into femoral artery

A similar increased permeability was observed after the retrograde infusion of Substance P into the femoral artery of four rats. Substance P at concentrations between 10^{-8} and 10^{-12} mol/l was infused at a rate of 0·24 ml/min for 5 min by a Beckman infusion pump and the skin of the lower paw examined. Blueing and carbon labelling of venules are present, the effect being visible at substance P concentrations as low as 10^{-11} mol/l (Fig. 2A).

Response to local application of capsaicin

Because capsaicin is thought to act predominantly by neural release of Substance P the effects of local application of capsaicin to hindlimb skin were studied. However, application of capsaicin to denervated skin caused some leakage of Evans' blue (Lembeck & Donnerer, 1981) and there is recent evidence that not only neurones containing Substance P are affected by capsaicin (Nagy *et al.*, 1981).

Local application of capsaicin (1% w/v solution in equal parts ethanol:DMSO) with a camel hair brush to the lower hind leg of four anaesthetized rats caused an immediate extravasation of Evans' blue starting within 10 minutes. Solvent alone had no effect. Carbon labelling studies show that leakage in this response occurs from both venules and capillaries. This response seen in Fig. 2B is indicative of direct endothelial damage.

Similar local application of capsaicin to four rats whose saphenous, sural and tibial nerves had been divided 7 days previously in an aseptic operation under nembutal anaesthesia, produced a reduced but still obvious increase in permeability. Again carbon labelling of both venules and capillaries was visible.

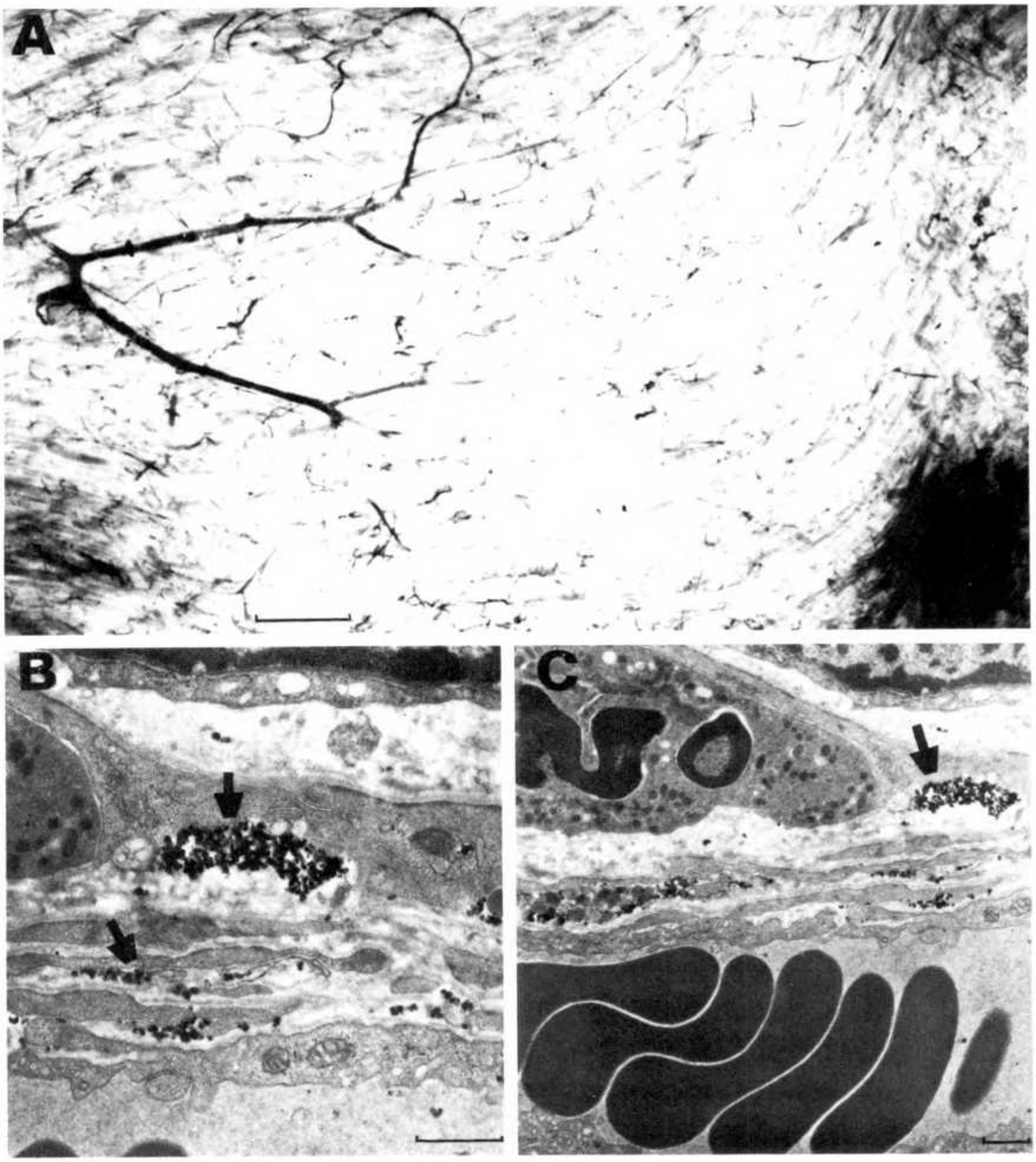

FIGURE 1. (A) Pattern of carbon deposition to antidromic stimulation of the saphenous nerve at C fibre strength. Cleared skin preparation from the rat hind leg in which microvessels are outlined in places by carbon. Scale bar = 300 μm. (B) and (C) Carbon deposits (arrows) in postcapillary venules to antidromic stimulation of a single polymodal nociceptive C fibre. EM sections of rat hind leg skin at magnification indicated by 1 μm scale bar.

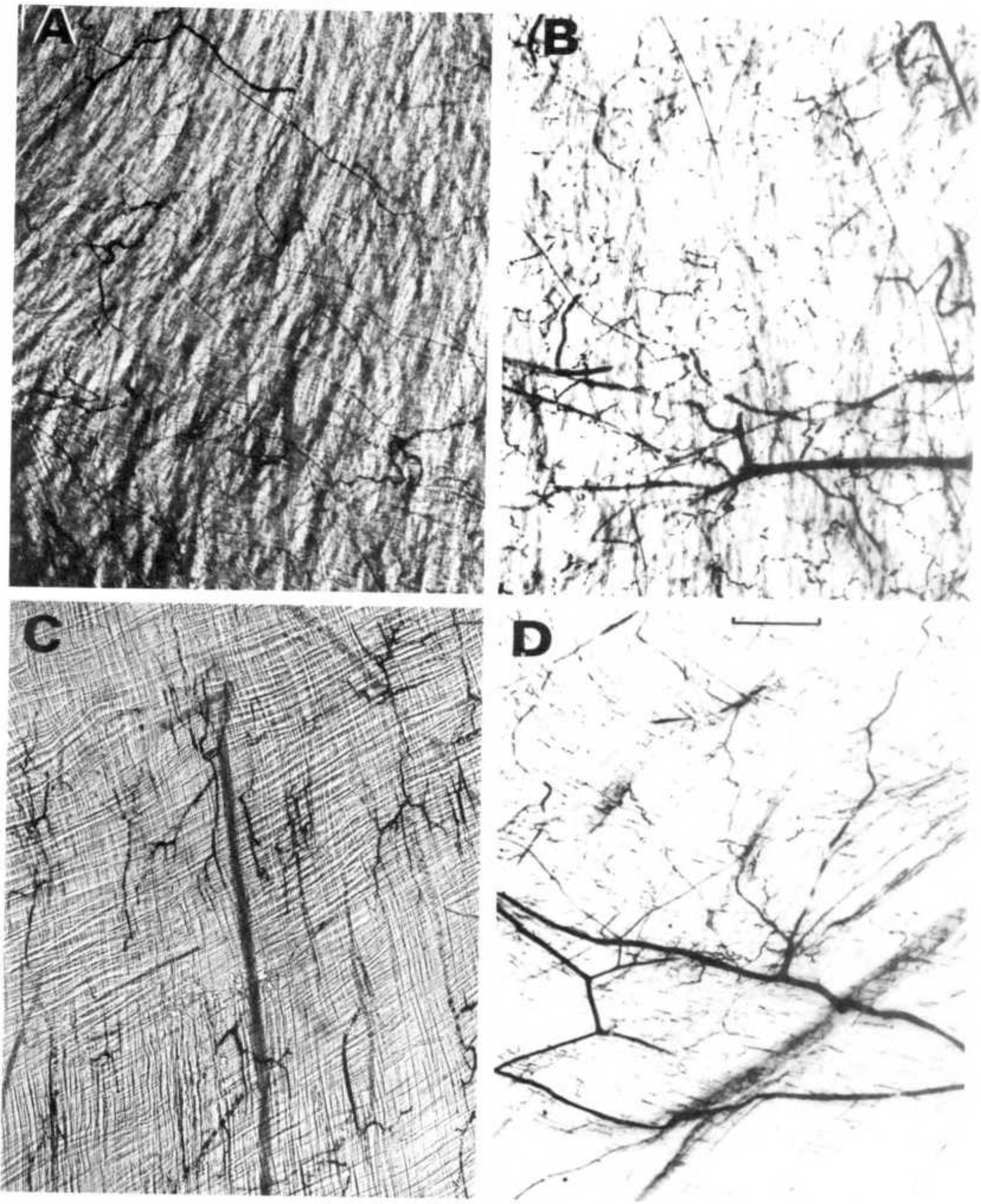

FIGURE 2. (A) and (B) Cleared skin preparations of carbon deposits in cutaneous blood vessels. (A) Response to Substance P retrogradely infused into the femoral artery at 10^{-8} mol/l. (B) Response to capsaicin as a 1% solution applied to the skin. Note the generalized leakage in (B). (C) and (D) Cleared cremaster preparations of carbon deposition. (C) Response to Substance P at 10^{-8} mol/l. (D) Response to capsaicin at 10^{-6} mol/l. Both substances locally applied. Note the direct damage type of vascular response in D. Scale bar 300 μm applies to (A), (B), (C) and (D).

Effects of local application of Substance P or capsaicin to the cremaster muscle

Because of its thinness and of the regular arrangements of its microcirculation, the cremaster muscle has been used widely for vascular labelling studies of the inflammatory response. It was decided to compare the effects of local application of substance P and capsaicin with the rat cremaster.

Either Substance P or capsaicin (four experiments of each) was injected in a volume 0·15 ml subcutaneously over the cremaster muscle, and cleared and EM specimens prepared by the same methods used for skin samples. Such local injection of capsaicin (10^{-6} mol/l) dissolved in olive oil produces an immediate increase in permeability and intense labelling of both venules and capillaries within the area in contact with capsaicin (Fig. 2D). Solvent alone was without effect.

By contrast, application of Substance P at a concentration of 10^{-8} mol/l produced immediate and short lived leakage in which labelling was confined strictly to venules and small veins (Fig. 2C). Pretreatment of two animals with a histamine antagonist (mepyramine maleate 1 mg/kg) 30 min before application of Substance P reduced but did not abolish the response.

DISCUSSION

By the technique of vascular labelling, the blood vessels with an increased permeability are outlined with the marker substance (colloidal carbon) at the actual site of increased permeability. The resulting pattern of carbon deposition (together with EM sectioning) establishes whether a mediator or a direct damage response has occurred (Majno & Palade, 1961; Cotran & Majno, 1964; Hurley, 1978). The cutaneous microvasculature is composed of a number of elements (arterioles, capillaries, postcapillary venules and collecting venules) each with a specialized function. Most inflammatory skin diseases (e.g. eczema and psoriasis) and all the known chemical mediators (histamine, bradykinin and serotonin) affect the postcapillary venules preferentially (Majno & Palade, 1961). The postcapillary venules are differentiated from the true capillaries by the presence of a multi-layered sheath of endothelial cells, and from the collecting venules by having a discontinuous layer of pericytes (for more details see Rhodin, 1968). In a mediator type of response discontinuous patches of carbon deposition are seen as only the postcapillary venules are affected. With direct damage all small vessels are affected and continuous lines of carbon coated vessels are seen in the affected area. For a more detailed description of the distinction between these lesions the article by Cotran & Majno (1964) and the review by Hurley (1978) should be consulted.

Our experiments show that the increased permeability evoked by antidromic stimulation of cutaneous nerves, and more particularly from the stimulation of sensory polymodal nociceptive C fibres, is of the mediator type suggesting a possible role for the nervous system in cutaneous inflammation.

Substance P is likely to be the mediator for neurogenic plasma extravasation for the following reasons. Intradermal or intra-arterial infusions of small amounts of Substance P evoke vasodilatation and plasma extravasation (Chahl, 1979; Lembeck & Holzer, 1979; Foreman *et al.*, 1983). Substance P is synthesized within the small ganglion cells of the primary afferent neurone of the dorsal root ganglion and transported both into the spinal cord, where it is found in grey matter laminae appropriate for a pain transmitter, and to the periphery, where it is found within nerve terminals which are often near blood vessels (Hökfelt *et al.*, 1975). Denervation leads to a substantial loss of cutaneous Substance P, as does local application of capsaicin (Lembeck & Holzer, 1979; Gamse, Holzer & Lembeck, 1980; Lembeck & Donnerer, 1981) which excites only polymodal nociceptive C fibres (Kenins, 1982). Stimulation of sensory nerves leads to the appearance of Substance P in the perfusate of the tissue (Olgart *et al.*, 1977) and release of Substance P from the spinal cord is in a manner consistent with its release being from neural stores (Otsuka & Konishi, 1976). Both neural stimulation and Substance P release histamine from mast cells (Erjavec *et al.*, 1981; Fewtrell *et al.*, 1982). Finally, some axon reflex effects can be blocked by Substance P antagonists (Rosell *et al.*, 1981; Lembeck, Donnerer & Bartho, 1982), although the specificity and extent of this antagonism is still to be determined. Thus the weight of this evidence argues for Substance P being both the sensory transmitter in pain pathways and the mediator for neurogenic plasma extravasation.

The vascular labelling and electron microscopy studies support this conclusion, as local or intra-arterial application of Substance P in low concentration (10^{-11} mol/l) produces a mediator type of leakage in the skin. Further evidence to support this conclusion was obtained from the rat cremaster muscle experiments. Antidromic stimulation of muscle nerves evokes vasodilation (Hilton, Hudlicka & Marshall, 1978) but no increase in vascular permeability (Bayliss, 1901). However, blood vessels in the cremaster do respond in the typical way to the local application of mediators (e.g. histamine), and the regularity of muscle vasculature as compared with that of the skin results in a clearer separation of the different types of permeability increase. These advantages have led to the use of the rat cremaster in many vascular permeability studies. Local application of Substance P to the cremaster muscle resulted in a mediator type of permeability increase at a concentration of 10^{-8} mol/l establishing Substance P to be as potent as bradykinin at increasing vascular permeability.

Thus our experiments together with the results of others mentioned above support the hypothesis that neurogenic plasma extravasation is evoked by polymodal nociceptive C fibres and is likely at least in part to be mediated by Substance P. The partial inhibition of the vascular effects of Substance P by prior administration of antihistamine compound confirms that Substance P acts in a large part by causing histamine release from local mast cells.

The mechanism by which the flare reaction spreads has been recently discussed (Foreman *et al.*, 1983). Evidence to support their suggestion of neurally mediated spread rather than the diffusable chemical model comes from the experiments of Helme & McKernan (1984). These authors found that capsaicin-induced erythema on the forehead in man does not spread across the midline. Although there is no barrier to diffusion across the midline of the forehead, nerves respect this boundary, as does the capsaicin-induced flare. Those results thus support the neurally mediated model of axon reflex erythema spread rather than spread by diffusion of a released chemical (e.g. histamine).

Capsaicin is a neurotoxin known to evoke plasma extravasation (Jansco, 1960) and to release substance P (Lembeck & Holzer, 1979). In our experiments local application of capsaicin to the skin resulted in a pattern of leakage not wholly of a mediator type, suggesting both a neurogenic and a direct toxic effect. Both capillaries and venules showed an increased permeability in a pattern similar to that produced by other agents known to cause direct endothelial injury (Hurley, 1978). In our experiments on the cremaster muscle (which is without neurally mediated plasma extravasation), local application of capsaicin evoked a direct injury type of response confirming the experiments on cutaneous leakage. A toxic action of capsaicin on all small neurons has been reported (Nagy *et al.*, 1981) and this general toxic action on both neurons and blood vessels independent of any neural action must be borne in mind in any physiological studies involving the use of capsaicin.

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Adrenoceptor-mediated responses in human skin studied by iontophoresis

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SUMMARY

The adrenoceptor-mediated responses in human skin (blanching and erythema) were studied by an iontophoretic technique. It is concluded that the receptors in superficial dermal blood vessels belong to the subclasses α_1 and β_2.

Iontophoresis, a process which causes increased penetration of ionized substances into tissues with the aid of an electric current, has been used in different situations both experimentally and therapeutically (Juhlin, 1961a; Harris, 1967; Hill, Gangarosa & Park, 1977; Gangarosa *et al.*, 1980). Blanching and pilomotion, the adrenergic responses to iontophoretically administered noradrenaline and adrenaline, have been studied and characterized in patients with atopic dermatitis (Juhlin, 1961a,b). Since then, our knowledge of adrenergic responses and receptors has profoundly increased. The α- and β-adrenoceptors have been divided into subclasses (α_1, α_2 and β_1, β_2 respectively) by using different *in vitro* experimental models (e.g. Exton, 1981; Kunos, 1978; Lefkowitz & Hoffman, 1980). The adrenoceptors seem to coexist in the same tissues in varying proportions depending on the tissue examined (Carlsson *et al.*, 1977; Daly & Levy, 1981; Wagner *et al.*, 1981). Although α- and β-adrenoceptors have been widely studied in a number of tissues, the skin and non-invasive techniques have attracted less attention. Therefore, we decided to use iontophoresis as a non-invasive tool to study adrenergic receptors and responses in human skin.

METHODS

The iontophoretic equipment used is a slightly modified copy of the one described in detail by Juhlin (1961a). It is constructed to give constant direct current, regardless of impedance. The anode is a multielectrode device. The current of each of the eleven chambers increases in proportional steps, i.e. approximately by $\sqrt{2}$, from 39 μA. Chamber no. 12 is used as a control and thus receives no current.

Healthy personnel and medical students served as volunteers (age range: 25–62 years). None of them was receiving medication which interfered with adrenergic responses. The flexural aspect of the forearm was gently wiped with ether. The multielectrode device was secured with rubber bands 10 cm below the antecubital fossa. The cathode, a brass metal plate approximately 22 cm^2, was covered with a thin foam rubber pad moistened with 0·9% NaCl solution and attached distally to the anode on the same arm. The multielectrode chambers were filled with an aqueous solution of the pharmacological agent. The electrode current was switched on for 30 seconds. The skin reactions—blanching, pilomotion or erythema—under the anode were read after 5 and 10 minutes unless otherwise stated. The experiments were performed at room temperature, 20–22°C, and before noon.

Drugs

Phenylephrine hydrochloride, isoprenaline chloride, yohimbine hydrochloride (Sigma Chemical Co., St Louis, Mo., U.S.A.), clonidine hydrochloride (Boehringer-Ingelheim AB, Sweden), prenalterol hydrochloride (Hyprenan®, Hässle AB, Sweden), terbutaline sulphate (Bricanyl®, Draco AB, Sweden), prazosin hydrochloride (Pfizer AB, Sweden), propranolol hydrochloride (ICI-Pharma AB, Sweden) were kindly supplied by the companies. Phentolamine (Regitin®, Ciba AB, Sweden) was prepared from a stock solution containing phentolamine-methane-sulphonate 10 mg/ml.

RESULTS

Blanching responses

Phenylephrine (α-agonist) caused blanching, which could be observed within 1 minute after termination of the iontophoresis (Fig. 1). The maximum blanching response occurred after approximately 5 minutes. As a rule blanching persisted for 30 minutes but often for even longer. Pilomotion was observed in many of the subjects but developed somewhat later than vasoconstriction, at the earliest after 2 minutes and usually only with higher current doses. Occasionally vasoconstriction and pilomotion persisted for 2 hours. Phentolamine (α-antagonist) markedly reduced the blanching and pilomotion induced by phenylephrine (Fig. 1). Yohimbine (α_2-selective antagonist) also reduced the blanching response of phenylephrine, but not so effectively as phentolamine (Fig. 1).

Clonidine (mainly α_2-agonist) induced blanching and pilomotion, both of which appeared somewhat later than with phenylephrine (Fig. 2). Phentolamine shifted the dose-response curve to the right. Yohimbine also reduced clonidine blanching at least with higher currents (Fig. 2).

The highly selective α_1-adrenoceptor antagonist prazosin could not be used due to the difficulty of dissolving it in water at pH 3·5 or higher. At this pH we produced urticarial reactions with water acidified with hydrochloric acid to pH 3.

Erythemal responses

The non-selective β-agonist isoprenaline induced erythema, which appeared after 2–10 minutes and was maximal between 10 and 20 minutes. The erythema was initially bright red and quite often patchy. As a rule the colour gradually became pinkish blue. In a few subjects an erythema persisted for as long as 35 minutes but was weaker (Fig. 3).

Propranolol, a non-selective β-antagonist, in concentrations between 1 mg/ml and 7·5 mg/ml, reduced the isoprenaline erythema (Fig. 3). In one subject the erythema was not only inhibited but gradually replaced by blanching with two of the highest currents.

The β_2-agonist terbutaline also produced an erythema but less pronounced than with isoprenaline. Prenalterol, a β_1-agonist, was without effect not only when administered by iontophoresis but also when injected intradermally (0·1 ml, 10 μg/ml) (Fig. 3).

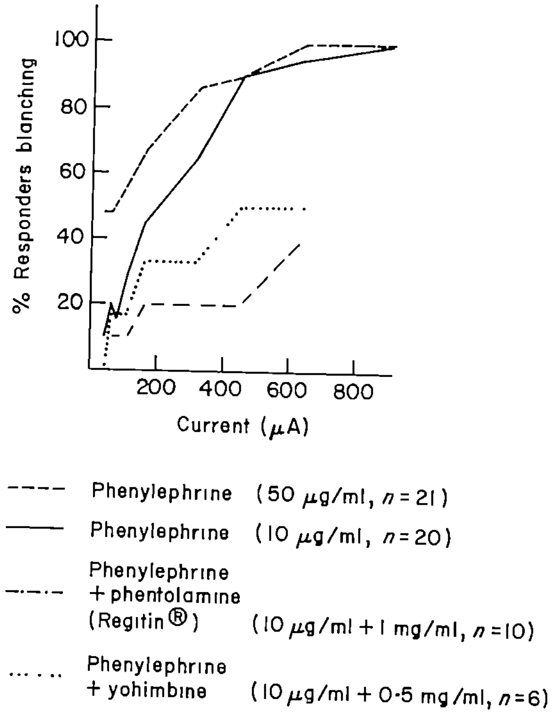

FIGURE 1. The effect of iontophoretically administered phenylephrine in two concentrations and with two different α-antagonists. Ordinate: Percentage of the subjects showing blanching. Abscissa: Minimal current in μA causing blanching. All six subjects tested with phenylephrine + yohimbine derive from the phenylephrine + phentolamine experiment ($n = 10$). The graphs are drawn from recordings made after 5 minutes for phenylephrine alone and after 10 minutes with the antagonists.

DISCUSSION

In humans α- and β-adrenoceptors have been identified in larger vessels. However, investigation of the cutaneous microcirculation has been hampered by methodological difficulties (Tharp, 1983). Skin reactions after intradermal injections of pharmacological substances may be hard to interpret, especially when studying erythema, since trauma to the tissue is produced. Also, it may be difficult to deposit the injected bolus exactly at the same level of the dermis.

The present study confirms previous studies indicating that iontophoresis is a non-invasive technique with good reproducibility for studying blood vessel reactions in human skin. It is rapid and painless. Graded responses are regularly recorded with increasing doses of the drug, i.e. the skin under the anode is paler or redder with the higher currents than with the lower ones (Juhlin, 1961a).

In our iontophoresis study we found that phenylephrine, which is a rather selective α_1-agonist, causes blanching (and often pilomotion) in all individuals provided that the current (i.e. dose) is sufficiently high. This accords with the findings for iontophoretically administered noradrenaline and adrenaline (Juhlin, 1961a). Clonidine imitated the blanching and arrector pili response of phenylephrine. However, the onset and maximum effect were delayed compared with phenylephrine. Phentolamine reduced the blanching and also the pilomotion responses evoked by both clonidine and phenylephrine. Yohimbine, which is more lipid soluble than

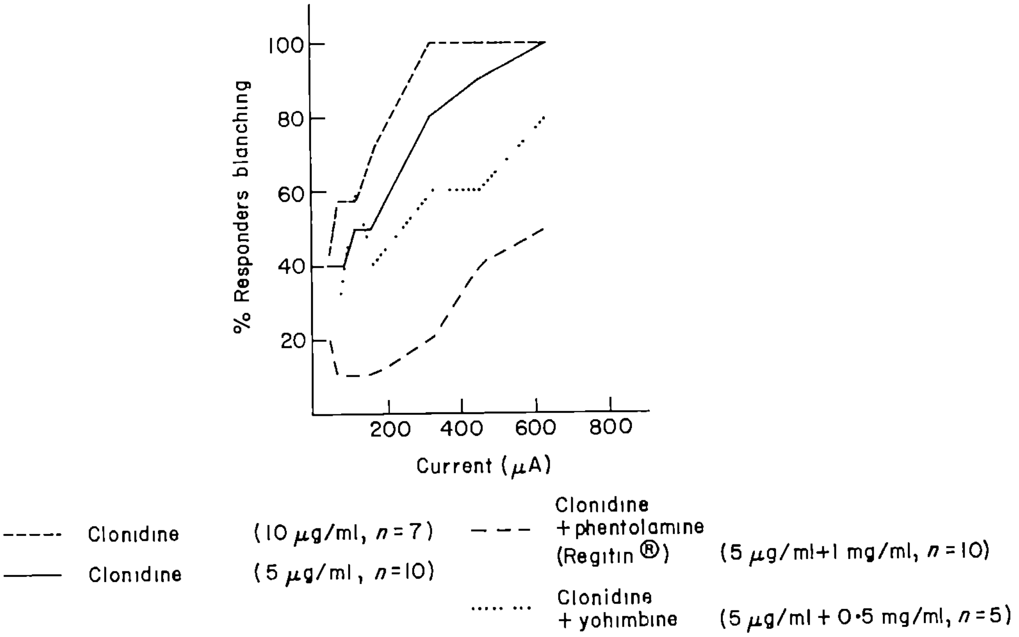

FIGURE 2. The effect of iontophoretically administered clonidine in two concentrations and with two different α-antagonists. All five subjects tested with clonidine + yohimbine derive from the clonidine–phentolamine experiment ($n = 10$). All graphs are drawn from recordings made after 10 minutes.

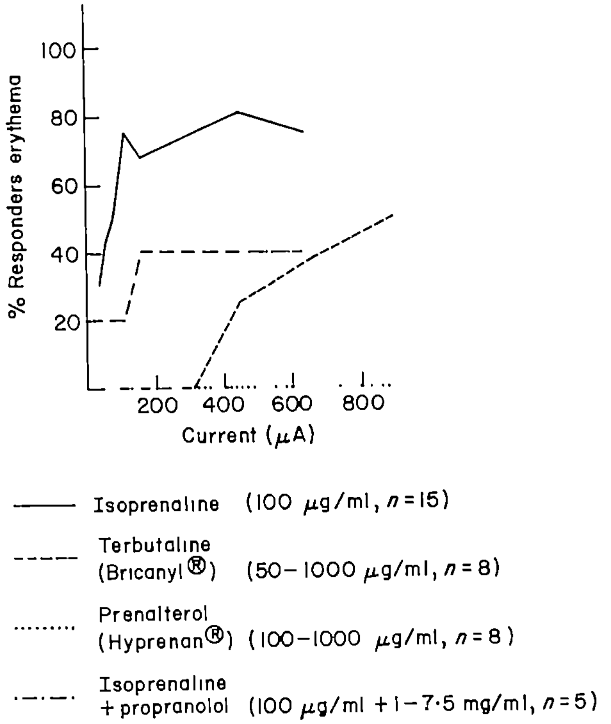

FIGURE 3. The effect of iontophoretically administered β-agonists and isoprenaline with a β-antagonist. All graphs are drawn from recordings made after 10 minutes.

phentolamine, also reduced the blanching responses but not to the same extent. Although clonidine is characterized as a rather selective α_2-agonist (Exton, 1981), it has actually been shown to act on both preganglionic and post-ganglionic α-adrenoceptors with variable potency in different organs (Houston, 1981). Furthermore, clonidine causes a 50–60% reduction of the resting skin blood flow immediately after i.v. administration (Merguet *et al.*, 1968). It is also known to provoke Raynaud's phenomenon when administered orally (Pykko & Färkkilä, 1982). Thus, these results indicate that the adrenoceptors mediating blanching and pilomotion mainly belong to the α_1-adrenoceptor subclass.

The non-selective β-agonist isoprenaline and the selective β_2-agonist terbutaline give rise to an erythemal reaction. This effect was clearly reduced by the β-blocker propranolol. The intensity of the erythemal reaction was subject to a large individual variation. The β_1-selective agonist prenalterol was without effect. These results strongly indicate that the erythemal reaction is mediated by the β_2-adrenoceptors.

In conclusion, iontophoresis is a non-invasive technique which could be used when studying adrenergic receptors in the skin of man and, as a result, might further delineate the proposed receptor defects in certain dermatological diseases and other pathological conditions as well. However, iontophoresis is beset by difficulties as far as interpreting the results are concerned, i.e. the concentrations of the various drugs at the receptor levels are not known due to differences in percutaneous penetration (*see* Scheuplein & Bronaugh, 1983). Binding to non-vascular tissue probably also influences the drug concentration at the receptor (Juhlin, 1961b).

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Effect of ultraviolet irradiation on granulocyte chemotaxis and nitroblue tetrazolium reduction activity in healthy individuals

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SUMMARY

The effects of ultraviolet irradiation on granulocyte chemotaxis and nitroblue tetrazolium reduction activities were studied in healthy volunteers. An increase of polymorphonuclear chemotaxis takes place after five irradiations, while the nitroblue tetrazolium reduction activity remains practically unchanged.

Both photo- and photochemotherapy are being used increasingly to treat an increasing number of skin diseases. Immunological effects at both experimental and clinical levels have recently been reported (Fox *et al.*, 1980; Greene *et al.*, 1979; Jansen, 1981), but these studies have mainly focussed on lymphocyte function and little is known about their effects on granulocyte function in healthy individuals.

In this study we present data on the effects of ultraviolet irradiation on the chemotaxis and nitroblue tetrazolium (NBT) reduction activities of peripheral blood polymorphonuclear leukocytes in healthy individuals.

METHODS

Eight healthy volunteers (two females and six males, aged 27–34 years) were included in the studies. They had received ultraviolet irradiation (Metec-Helarium 1480/2, Metec Co. Munich, Germany, radiation spectrum: 290–400 nm) five times in a period of 10 days (total dose: 20 joules/cm^2).

The chemotactic and the NBT reduction activities of the granulocytes were determined before and 16–20 h after the last whole body irradiation. The granulocytes were separated from the fresh venous blood by dextran sedimentation (Csato, Dobozy & Simon, 1980). The

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chemotactic activity of the granulocytes was evaluated with a Boyden chamber method as described earlier (Csato *et al.*, 1981). Zymosan-activated pooled healthy donor serum was used as chemo-attractant. A quantitative method was performed to estimate the zymosan-stimulated NBT reduction activity of the polymorphonuclear leukocytes (Csato *et al.*, 1980).

Statistical calculations were carried out with the Student's paired *t*-test.

RESULTS

As presented in Fig. 1, the chemotactic responsiveness of the granulocytes is significantly enhanced following five irradiations. The alterations of the NBT reduction activity are insignificant (Fig. 2).

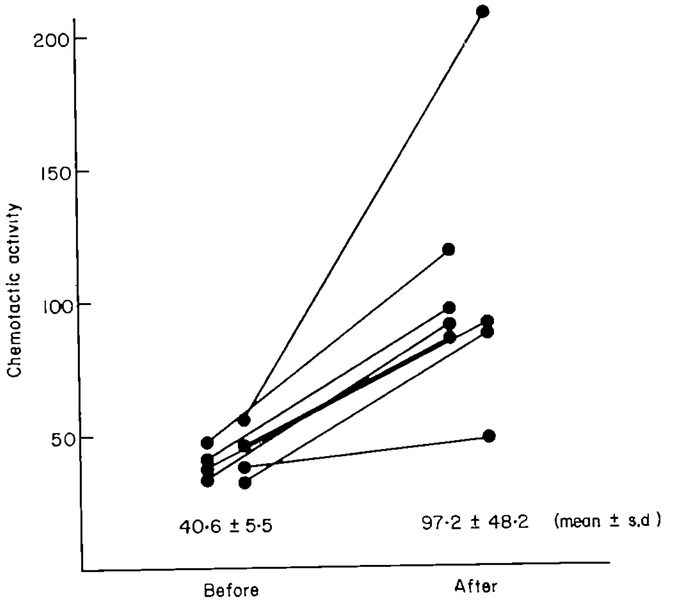

FIGURE 1. Chemotactic activity of polymorphonuclear leukocytes before and after ultraviolet irradiation in healthy individuals. The chemotactic activity is designated as the mean number of granulocytes counted microscopically on the lower side of the filter in the Boyden chamber. Student's paired *t*-test was used for statistical evaluation ($P < 0{\cdot}02$).

DISCUSSION

So far as we know, the effects of ultraviolet irradiation have not been previously studied on the peripheral blood granulocyte functions *in vivo* in healthy individuals. Our data indicate that a significant increase in granulocyte chemotaxis takes place after ultraviolet irradiation in healthy persons, but the alterations of the NBT reduction activity are inconsequential.

Eaglstein, Sakai & Mizumo (1979) found that a significant granulocyte immigration into the skin occurred in guinea-pigs after UV-B irradiation with a peak at 4–16 h after the treatment. Furthermore, UV-B response was diminished in animals rendered leukopenic by cyclophos-

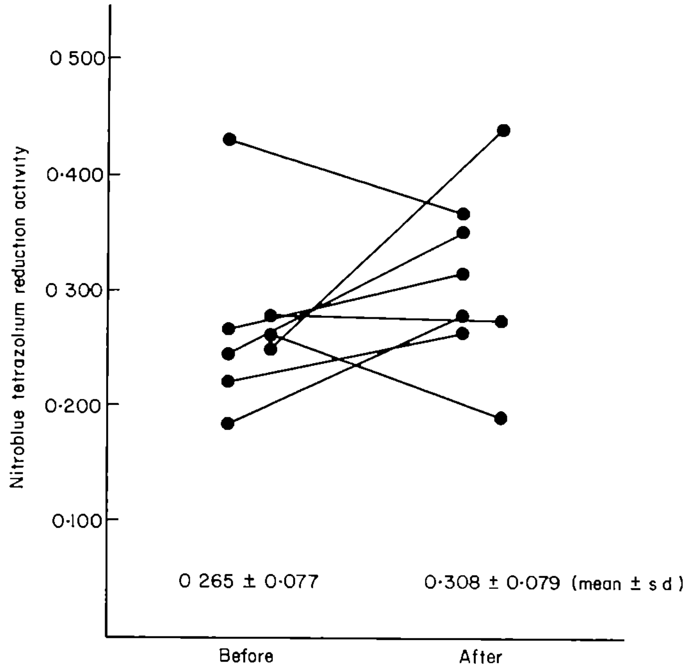

FIGURE 2. Nitroblue tetrazolium reduction activity of polymorphonuclear leukocytes before and after ultraviolet irradiation in healthy individuals. The nitroblue tetrazolium reduction activity, i.e. the formazan resulted from the reaction was measured photometrically and is expressed in optical density units. Student's paired *t*-test was used for statistical calculation ($P = 0{\cdot}20$).

phamide pretreatment, while the UV-A and UV-C erythema reactions were not influenced. The number of circulating granulocytes, but not lymphocytes, has been shown to increase following UV-B irradiations in rats (Spode, 1974). These observations point to the importance of polymorphonuclear leukocytes in UV-B-induced inflammation. Langner & Christophers (1977) demonstrated a decrease in normal human polymorphonuclear chemotaxis after PUVA treatment in *in vitro* studies. The light source which we have used has a broad emission spectrum including a significant amount of UV-B radiation. Therefore, we assume that UV-B accounts for the chemotaxis enhancement in healthy individuals.

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Desmoplastic trichoepithelioma: clinicopathological features and immunohistochemical study of the basement membrane proteins, laminin and type IV collagen

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SUMMARY

Six desmoplastic trichoepitheliomas (DT) from four patients were studied immunohistochemically, using antibodies to the human P1 fragment of laminin and the 7S domain of type IV collagen. The staining of the basement membrane around the tumour clusters was distinct and continuous in most areas, but there were discontinuities and a granular appearance of the basement membrane in the areas of keratinized cysts and calcification around some unorganized epithelial cell nests. The discontinuities of the basement membranes may be a sign of epithelial degeneration or altered differentiation rather than malignancy.

Desmoplastic trichoepithelioma (DT) is a recently described adnexal tumour entity, although it has been known since the beginning of this century in the form of solid epithelioma adenoides cysticum (Wolters, 1901; Schopper, 1909). It shows a close histological resemblance to morphoeic (fibrosing) basal cell carcinoma, conventional trichoepithelioma and syringoma. It differentiates towards a hair follicle or sweat or sebaceous duct and has been tentatively regarded as benign (Brownstein & Shapiro, 1977; MacDonald, Wilson Jones & Marks, 1977; Lever & Schaumburg-Lever, 1983). Its differential diagnosis still causes difficulty to both dermatologists and pathologists. Clinically DT most often occurs as an asymptomatic yellowish, depressed, non-ulcerated lesion with raised annular borders on skin exposed to the sun, particularly on the faces of young and middle-aged females (Brownstein & Shapiro, 1977; MacDonald *et al.*, 1977; Lever & Schaumburg-Lever, 1983). The clinical and histological findings can easily lead to misdiagnosis as basal cell carcinoma.

The role of the basement membrane (BM) in benign and malignant neoplasia has been

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intensively investigated (Albrechtsen *et al.*, 1981; Barsky *et al.*, 1983; Birembaut *et al.*, 1983; Nielsen, Christiansen & Albrechtsen, 1983; Kallioinen *et al.*, 1984a). Type IV collagen, which is mainly located in the lamina densa, forms the major component of the BM. It is organized in a net-like fashion with four molecules linked together at their N-termini to form the 7 S domain of type IV collagen (Risteli *et al.*, 1980; Timpl *et al.*, 1981). Laminin is one of the non-collagenous glycoproteins of BM, the others being entactin, heparin sulphate proteoglycan and possibly fibronectin. These proteins are mainly located in the lamina lucida (Stanley *et al.*, 1982b; Martinez-Hernandez & Amenta, 1983).

The BM is intact in many benign skin tumours, while it is absent or extensively disrupted in their malignant counterparts (Kallioinen *et al.*, 1984b). Exceptions among the malignant skin tumours are basocellular carcinomas (BCC), in which the BM is usually distinct and regular (Stanley *et al.*, 1982a; Weber *et al.*, 1982; van Cauwenberge *et al.*, 1983; Nelson, Little & Balian, 1983; Kallioinen *et al.*, 1984a). Fibrosing BCCs, however, show disruptions in the BM, probably reflecting the more aggressive nature of these tumours (Kallioinen *et al.*, 1984a).

The purpose of this study was to compare clinical and histological findings from six desmoplastic trichoepitheliomas with previous descriptions and to investigate the occurrence and nature of the laminin and type IV collagen components of the BM in these tumours.

METHODS

Six desmoplastic trichoepithelioma biopsy samples from four patients were fixed in 10% phosphate-buffered formalin and embedded in paraffin. Sections 5 μm in thickness were stained with haematoxylin and eosin for light microscopy. Detailed data on the patients are presented in Table 1.

Antibodies to the human 7S collagen domain of type IV collagen and human P1 fragment of laminin were kindly donated by Drs J. and L. Risteli. The purification and characterization of the antigens and the preparation of the antibodies have been described earlier (Risteli *et al.*, 1980; Risteli & Timpl, 1981).

TABLE 1. Clinical features

Case	Sex	Age	Duration of the tumour before excision	Site(s)	Appearance	Initial clinical diagnosis	Initial histological diagnosis
1	F	14	Several years	Chin	Atrophic lesion, 5 × 5 mm	Lichen sclerosus et atrophicus	Trichoepithelioma
2	F	21	Several years	Cheek	Central depression, raised edges, 6 mm in diameter	Naevus sebaceous	Syringoma
3	F	24	7–8 years	Cheek	As above, 5 × 7 mm	DT	DT
4*	F	40	20–25 years	Cheek	As above, 10 × 10 mm	BCC	Basalioma, morphoea-type
5*				Right corner of mouth	As above, 3 × 3 mm	BCC	Syringoma
6*				Back	As above	BCC	Syringoma

* Same patient.

The sections for immunohistochemical staining were deparaffinized and treated with 0·4% pepsin (Chemical Co, St Louis, MO) to enhance the availability of the antigens (Burns, Dixon & Woods, 1980; Ekblom *et al.*, 1982). Endogenous peroxidase was inactivated by exposing the sections to 0·1% hydrogen peroxide and the peroxidase–antiperoxidase procedure was then performed according to the method of Sternberger (Sternberger, 1979). Normal rabbit serum and phosphate-buffered saline were used as control substances in place of the primary antibody.

RESULTS

Clinical findings

The clinical findings and initial diagnoses are presented in Table 1. All the patients were females, aged 14–40 years. The typical lesion was a tumour with a central depression and raised edges (Fig. 1). One patient had three DTs (cases 4–6), one of which was on the back. The youngest patient (case 1) had also lichen sclerosus et atrophicus (LSA) lesions on her skin, and consequently the lesion on her chin was thought to be LSA.

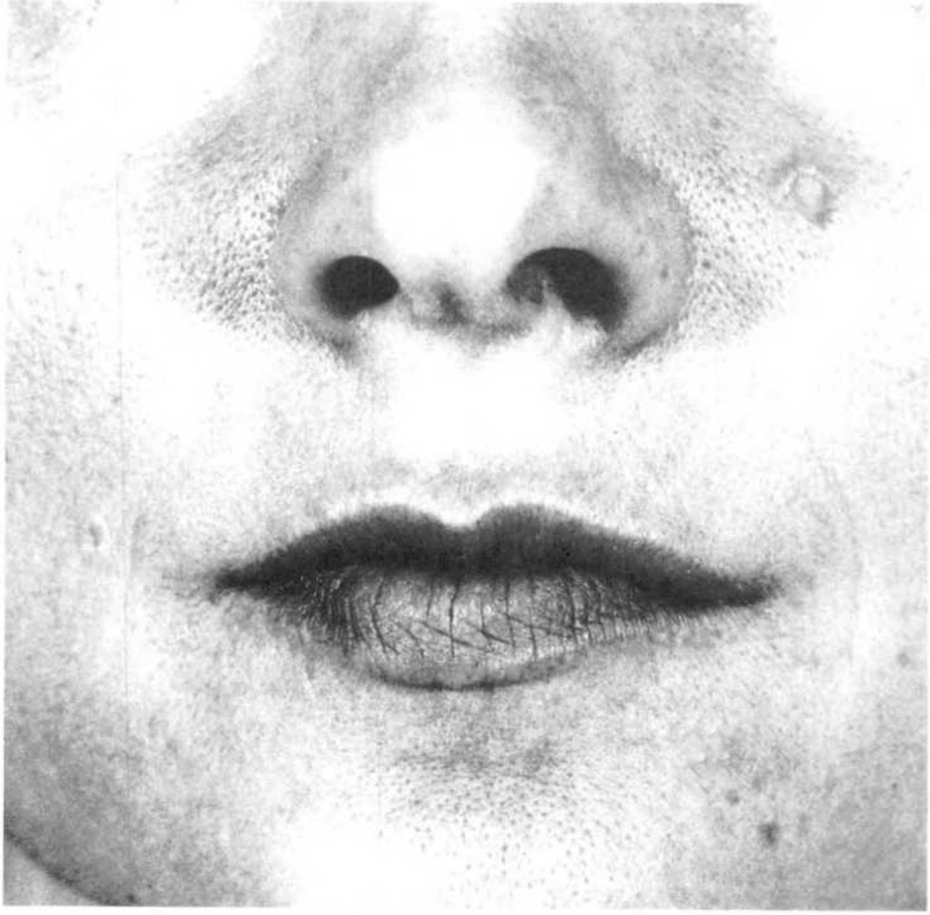

FIGURE 1. Desmoplastic trichoepitheliomas on the face of a 40-year-old woman. The picture shows a 3 × 3 mm depressed lesion with raised edges in the right hand corner of the mouth and a 10 × 10 mm crateriform lesion on the left cheek (cases 4 and 6).

Histology and immunohistochemistry

All six DTs were composed of narrow strands of basaloid tumour cells and horn cysts within a desmoplastic stroma. Each contained areas of calcification (Fig. 2). The tumour cells were small with little cytoplasm. The nuclei were round or oval and the chromatin was finely granular and evenly distributed (Fig. 3). The amount and distribution of the desmoplastic stroma were similar in all the tumours.

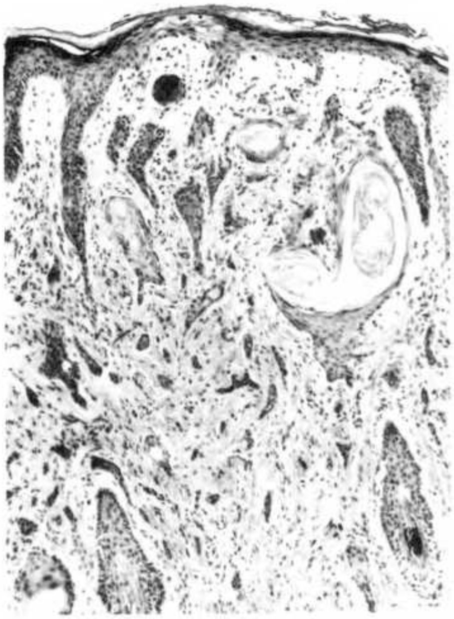

FIGURE 2. A desmoplastic trichoepithelioma. Tubular structures, partly keratinized, solid epithelial sheets and a calcified focus are seen within desmoplastic stroma (case 1). (H & E, magnification × 40.)

The BM around the tumour strands stained positively for both laminin and type IV collagen in all the DTs, and there was no intracellular staining in any case. The BMs of all the tumours stained comparably with laminin and type IV collagen antibodies. The BM was distinct and continuous in most parts of the tumours, but showed disruptions or even complete absence around the large keratin cysts and at the foci of calcification (Fig. 4). Some solid, poorly organized tumour cell clusters were surrounded by uneven, granular BM material (Fig. 3).

The staining of the BM of the normal epidermis, appendigeal structures and dermal capillaries was distinct in every case (Fig. 4) and all the control stainings with normal rabbit serum and phosphate-buffered saline were negative.

DISCUSSION

Desmoplastic trichoepithelioma has been recognized as a separate entity among the adnexal tumours since 1977 (Brownstein & Shapiro, 1977; MacDonald *et al.*, 1977). It is said to be as common as trichofolliculoma and eccrine spiradenoma, but seven times less common than

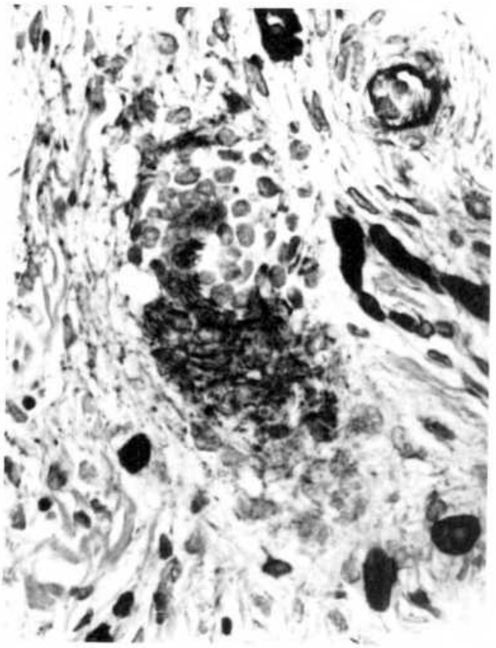

FIGURE 3. Immunostaining of a desmoplastic trichoepithelioma for type IV collagen. Granular BM material is seen around the cells of a solid epithelial nest, possibly an immature neoplastic tubulus. The BM is linear around the capillaries and well-formed tubuli. The nuclei of the tumour cells are regular (case 4). (Magnification × 248.)

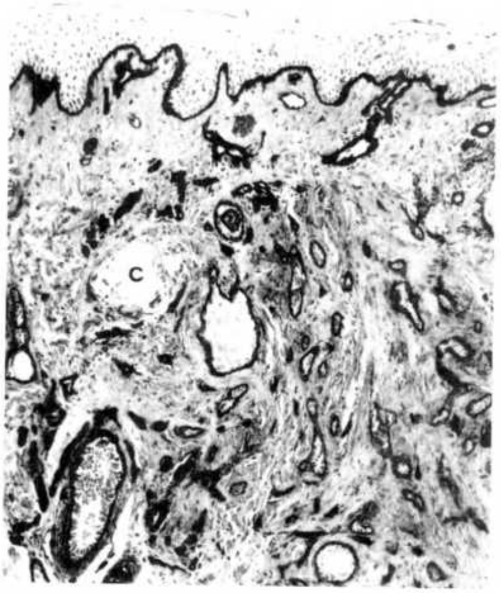

FIGURE 4. Immunostaining of a desmoplastic trichoepithelioma for type IV collagen. The BM is continuous and distinct beneath the normal epidermis around the follicles, the dermal capillaries and most neoplastic epithelial tubules and sheets. A degenerated keratinized cyst (c) shows a lack of BM (case 1). (Magnification × 48.)

conventional trichoepithelioma (Brownstein & Shapiro, 1977). The present six DTs found in four patients during a 3-year period suggest that this is a relatively common tumour.

The clinical and histological findings in our cases were in good agreement with previous descriptions (Brownstein & Shapiro, 1977; MacDonald *et al.*, 1977).

The basement membrane was intact in most parts of the DTs, and the defects around the keratin cysts and calcified areas were identical with those found in ordinary trichoepitheliomas, trichofolliculomas, and pilomatrixomas (Kallioinen *et al.*, 1984b). The epithelial cells of such areas are probably too differentiated or degenerated to be able to produce or maintain a BM. This would be analogous to the situation in the normal epidermis, where the basal cells but not the squamous cells are capable of synthesizing BM. The uneven, granular BM material around some poorly organized cell nests may represent an immature stage in the neoplastic process comparable to primitive renal glomeruli, which also contain extracellular granular laminin and type IV collagen prior to developing into morphologically mature structures (Ekblom *et al.*, 1980). Identical unorganized cell nests with granular BM material around them have been reported in syringomas (Kallioinen *et al.*, 1984b). It is not known whether DT differentiates towards sweat or sebaceous ducts or hair follicles (Brownstein & Shapiro, 1977). Immunostaining for laminin and type IV collagen has not helped to discriminate between the various adnexal tumours (Kallioinen *et al.*, 1984b).

The term 'desmoplastic' emphasizes the rich fibrotic stroma of the tumour. In morphoeic basal cell carcinomas the fibrotic stroma, together with intermingled stromal and epithelial cells (Mehregan, 1983), have been regarded as indicative of a more aggressive behaviour (Sloane, 1977; Jacobs, Rippey & Altini, 1982; Mehregan, 1983). Thus we have found the BM defects in fibrosing BCCs to be more advanced than in DTs (Kallioinen *et al.*, 1984a). The extent of stromal desmoplasia in the present DTs seemed to have no effect on the BM. BM fragmentation and its total absence from wide areas is generally associated with a malignant tumour (Albrechtsen *et al.*, 1981; Barsky *et al.*, 1983; Birembaut *et al.*, 1983; Nielsen *et al.*, 1983), but an intact BM does not exclude the possibility of malignancy (Stanley *et al.*, 1982a; Weber *et al.*, 1982; Nelson *et al.*, 1983; van Cauwenberge *et al.*, 1983; Kallioinen *et al.*, 1984a).

The results suggest that a DT is usually a benign tumour. The discontinuities of the BM in some tumour areas may not indicate malignancy but be a sign of altered epithelial differentiation or degeneration. Follow-up studies on larger series are needed, however, before the grade of malignancy of DT can be established with certainty.

ACKNOWLEDGMENTS

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Cytogenetic studies in a patient with porokeratosis of Mibelli, multiple cancers and a *forme fruste* of Werner's syndrome

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SUMMARY

A 49-year-old man with extensive porokeratosis of Mibelli (PM) developed a squamous cell carcinoma and several carcinomas-*in-situ* within the lesional skin. The patient also had diabetes mellitus and a short stature with a prematurely aged appearance. The patient's father and two siblings also had PM. The patient died from metastatic squamous cell carcinoma, and at autopsy an adenocarcinoma of the descending colon was also found.

Fibroblasts cultured from both the PM-affected and unaffected skin showed chromosomal abnormalities and a decreased lifespan. Cellular sensitivity to ultraviolet rays measured by unscheduled DNA synthesis and colony-forming ability were within normal limits. An association with a *forme fruste* of Werner's syndrome was suspected.

Porokeratosis of Mibelli (PM) shows characteristic skin lesions with brown, keratotic papules which gradually enlarge to form annular lesions with atrophic centres. A photosensitive variety, disseminated superficial actinic porokeratosis (DSAP) has been separated from PM (Chernosky & Freeman, 1967), and ultraviolet radiation (UVR) provokes typical porokeratotic lesions in DSAP (Chernosky & Anderson, 1969).

We describe a patient with PM who developed carcinomas of the skin and the descending colon. Cytogenetic studies were performed to observe the effects of UVR in this patient.

CASE REPORT

A 49-year-old Japanese male plasterer was seen because of an ulcerated tumour on the dorsum of the left foot. Brownish spots were first found on the limbs at the age of about 13 years. These gradually increased in number, and covered almost the entire surface of the body except for the

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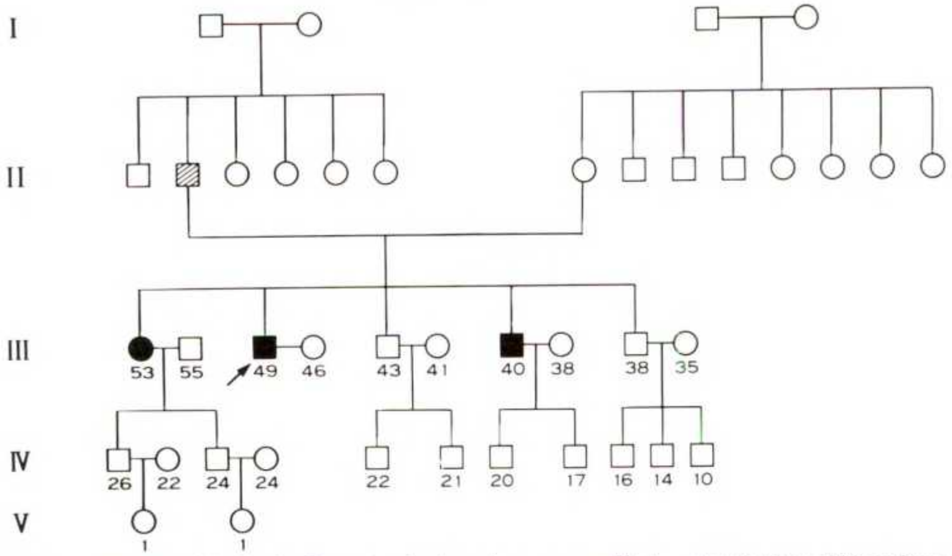

FIGURE 1. Pedigree of the family. Propositus is shown by an arrow. Black symbols indicate PM confirmed by examination, and shaded symbols only by history. Number corresponds to age of the subject.

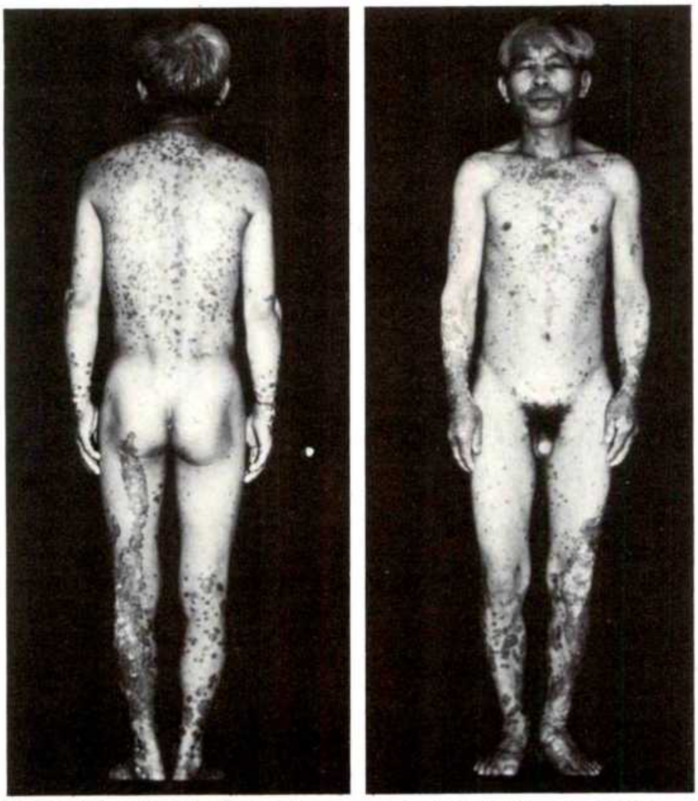

FIGURE 2. Frontal and dorsal view of the patient showing short stature, premature greying of hair and extensive porokeratosis lesions.

palms and soles by the age of 40 years. The father, an elder sister and younger brother had similar but less extensive brown skin lesions (Fig. 1). There was no consanguinity in the parents. The patient had been married for 24 years but was childless.

Physical examination revealed a short, elderly-looking Japanese male with moderate greying of the scalp hairs. The body weight was 43 kg and the height was 149 cm. Numerous round or annular brownish macules were symmetrically distributed over the entire body. There were coalescent, larger macules forming plaques on the dorsa of hands, distal portions of the forearms and along the left foot (Fig. 2). Thick keratosis covered the centres of the larger plaques. A 6·0 × 5·5 cm, ulcerated mass was found within the brownish plaque on the dorsum of the left foot (Fig. 3). The palms, soles and visible mucosae were not involved. A 1·5 × 1·5 cm, elastic, hard, left inguinal lymph node was palpable. The testes were atrophic, and there were no cataracts.

Routine laboratory tests (full blood count, etc.) were within normal limits, except for elevated fasting blood sugar of 127 mg/dl, alkaline phosphatase of 194 i.u./l, lactate dehydrogenase of 122 i.u./l and γ-glutamyl transpeptidase of 108 i.u./l. Serum IgG was 908 mg/dl, IgA 195 mg/dl, IgM 19 mg/dl, IgD 2·8 mg/dl and IgE 315 U/ml. Serum insulin, TSH, carcinoembryonic antigen, α-fetoprotein, and urinary 17-OHCS and 17-KS levels were also within normal limits. Oral 50 g glucose tolerance test showed a diabetic pattern.

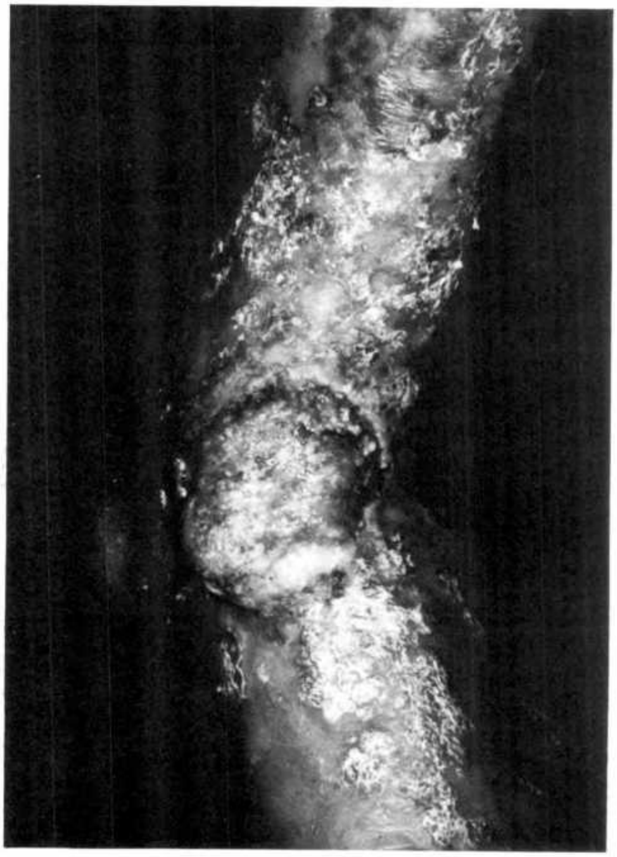

FIGURE 3. Ulcerated cauliflower-like mass within scaly brownish plaque on the dorsum of the left foot.

Intracutaneous reactions with purified protein derivatives of tuberculin produced 10 × 10 mm erythema, and with streptokinase-streptodornase 3 × 4 mm erythema. Percutaneous sensitization with dinitrochlorobenzene was successfully produced. Minimal erythema dose (MED) to UV-B (2·5 mW/cm^2 at 305 nm, FL-20S·E30 lamp, Toshiba) was $4{\cdot}5 \times 10^4$ J/m^2, which was not decreased. Photoprovocation with UV-B of $2{\cdot}7 \times 10^6$ J/m^2 or with UV-A of $1{\cdot}8 \times 10^5$ J/m^2 (25 mW/cm^2 at 365 nm, FL-20·BLB lamp, Toshiba) over topical 0·3% 8-methoxypsoralen in alcohol produced only erythema followed by thin brownish pigmentation. Both UV-B and UV-A energies were measured by a radiometer (UVR-305/365, Torex).

Roentgenograms of the chest, gastrointestinal tract, vertebrae and extremities showed no abnormalities. ^{67}Ga- or ^{99}Tc^{m}-labelled complexes showed abnormally high uptakes over the left foot where the ulcerated mass was located. Inguinal lymphangiography revealed enlarged external iliac and para-aortic lymph nodes.

Haematoxylin–eosin stained sections from the brownish macules on the trunk showed typical cornoid lamellae of PM and those from scaly, slightly raised plaques on the right hand and on the knees showed moderate acanthosis with a disorderly arrangement of atypical epidermal cells. The dermal papillae were densely infiltrated by lympho-histiocytic inflammatory cells. The sections from the ulcerated mass on the dorsum of the left foot revealed invasive squamous carcinoma.

The squamous carcinoma of the foot was excised *in toto* followed by a skin graft and radical dissection of the left inguinal and retroperitoneal lymph nodes, which showed metastases to the L_2 level. Intramuscular injection of bleomycin 15 mg per day twice weekly totalling 60 mg was given. The scaly plaques of Bowen's disease were treated with topical 5% 5-fluorouracil ointment under occlusion. The left foot tumour recurred 3 months later and was again removed surgically. Subcutaneous nodules appeared subsequently in the left inguinal region, which was treated with 6000 rad (60 Gy) of ^{60}Co gamma-rays. The patient died 22 months after the admission.

The autopsy revealed generalized metastases of squamous cell carcinoma, which were also found in the skin of the left knee and inguinal area as well as in many internal organs. A well-differentiated adenocarcinoma of the descending colon was found with no evidence of metastasis.

CYTOGENETIC STUDIES

Preparation of fibroblast strains

Biopsies were taken from PM-affected skin of the inner aspect of the left arm and from unaffected skin of the back before treatment. The tissues were cut into fragments and were inoculated into a tissue culture flask (25 cm^2) containing 1 ml of Eagle's minimal essential medium supplemented with 15% fetal bovine serum and 60 mg/l of kanamycin. The flasks were incubated in 5% CO_2 in air at 37°C. The medium was changed twice a week until fibroblast outgrowth covered 1/3 of the flask, when the medium was removed and the cells were rinsed with phosphate-buffered saline (PBS) followed by vigorous pipetting with 0·5 ml of 0·25% trypsin and 0·02% EDTA for a few minutes. When they become confluent, whole cells were trypsinized and transferred to a 75 cm^2 flask with 15 ml of the medium. Subsequently, the medium was changed twice a week until confluency and they were harvested. Then 10^6 cells were seeded on to a new flask or stored in the medium containing 10% dimethylsulphoxide at −80°C until use. Every experiment was duplicated at least twice.

Table 1 shows the cell strains used in comparison with PM cells. XP3OS (Takebe *et al.*, 1977)

TABLE 1. Fibroblast strains used in the cytogenetic studies and their UDS levels and chromosomal abnormalities

Strain	Donor				UDS (Mean ± s.e.)	Chromosomal abnormalities
	Sex	Age	Disorder	Site		
PM1EH$_n$	Male	49	PM	Unaffected	73·2 ± 1·6	8/50 cells
PM1EH$_p$	Male	49	PM	Affected	68·1 ± 1·2	6/50 cells
PM2EH	Female	45	PM	Affected	65·5 ± 0·9	0/50 cells
PM3EH	Female	72	PM	Affected	56·0 ± 0·8	0/50 cells
DP1EH	Male	52	DSAP	Affected	74·4 ± 1·4	n.d.
XP1EH	Male	10/12	XP	Unexposed	6·5 ± 0·4	n.d.
XP3OS	Female	5	XP	Unexposed	2·2 ± 0·2	n.d.
KAIS	Male	31	Normal	Unexposed	67·1 ± 1·7	0/50 cells
MAKA	Male	45	Normal	Unexposed	75·6 ± 1·8	0/50 cells

n.d. = not done.

was given by Dr Takebe of Kyoto University. The other strains were prepared by us. PM2EH and PM3EH were derived from other untreated PM patients. Both XP1EH and XP3OS from xeroderma pigmentosum patients were used as DNA repair deficient controls. DP1EH was derived from DSAP which was clinically more UV-sensitive than PM. KAIS and MAKA were normal controls.

Population doubling number (PDN)
This was calculated by the following formula (Ban, Nikaido & Sugahara, 1980):

$$\mathrm{PDN}_n = \log_2\left(\prod_{k=1}^{n} G_k\right)$$

where G_k = growth rate, and n = nth passage.

Unscheduled DNA synthesis (UDS) and colony-forming ability (CFA). UDS and CFA after 254 nm UV irradiation were examined by procedures described previously (Yagi, 1982).

Chromosome analysis. The chromosomes were analysed in the early passages by the procedures described by Sasaki *et al.* (1980).

RESULTS

PDN of PM1EH$_p$ was remarkably decreased compared with those of PM2EH and normal controls, indicating a shorter lifespan of the cultured fibroblasts in the present patient (Fig. 4). The levels of UDS in PM cells were not reduced (Table 1). CFA was not affected after UV irradiation (Fig. 5). The UV sensitivities of PM1EH$_p$, PM2EH and PM3EH were the same as in normal controls. Increased chromosomal abnormalities were found in both PM1EH$_p$ and PM1EH$_n$ derived from PM-affected and unaffected skins of the patient (Table 1). Abnormalities were mostly those of translocations, the site of which were at random, and gaps and breaks were not increased compared with normal controls.

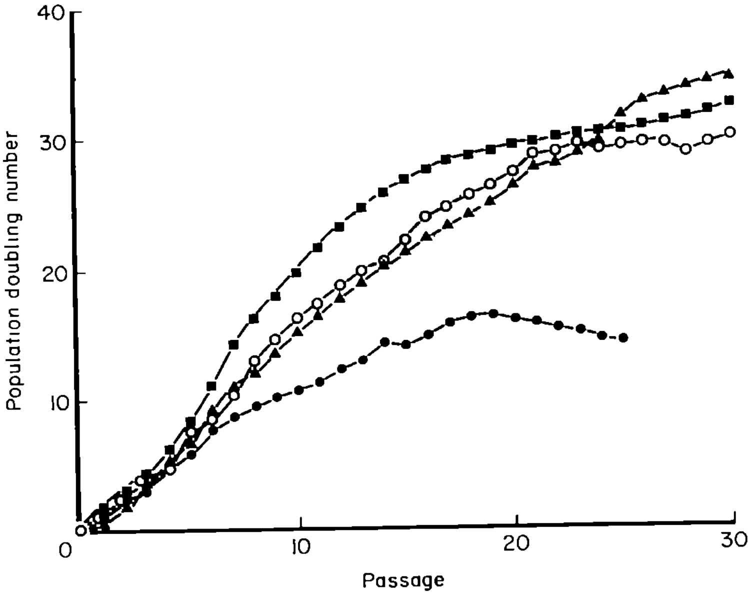

FIGURE 4. Population doubling number of fibroblast strains. (●) PM1EH$_p$; (○) PM2EH; (▲) MAKA; (■) KAIS.

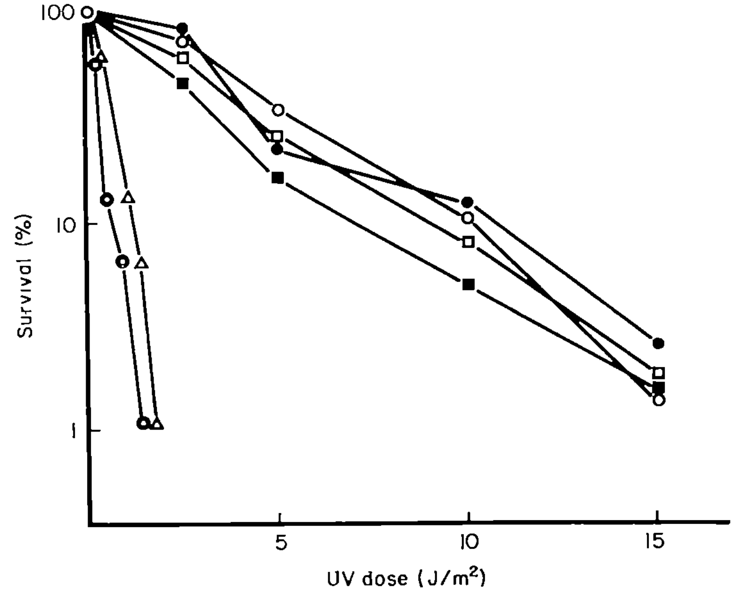

FIGURE 5. Survival of fibroblast strains following UV irradiation. (■) KAIS; (●) PM1EH$_p$; (○) PM2EH; (□) PM3EH; (△) XP1EH; (⊙) XP3OS.

DISCUSSION

PM is frequently inherited as an autosomal dominant trait. The male to female ratio is 3 to 1 (Goerttler & Jung, 1975). Skin eruptions usually begin by middle age and 7% of the patients ultimately develop skin cancers. The cancers have been limited almost exclusively to the extremities except in three cases (Goerttler & Jung, 1975), and the risk of developing malignancy appears to be greater in sporadic rather than in familial cases (Cort & Abdel-Aziz, 1972). Quiescent PM lesions became active and extensive following an immunosuppressive treatment for renal transplantation (MacMillan & Roberts, 1974). DSAP lesions are known to be provoked by UV rays (Chernosky & Anderson, 1969). It seems that both porokeratosis and skin cancer may be triggered by minor trauma, UVR, X-rays or immunosuppression.

In the present patient, cellular and humoral immunities were not apparently impaired when he presented. He had diabetes mellitus and short stature with prematurely aged appearance and atrophic testes. The MED was not decreased, and 60 MED of UV-B failed to produce porokeratosis lesions in unaffected area. The development of squamous cell carcinoma and carcinomas-*in-situ* in the present patient may have been the result of long-standing UVR exposure of over 30 years' duration.

Cytogenetic studies on disorders with susceptibility to neoplasms have recently opened a new field of cancer research (Setlow, 1978). In recessively inherited, deficient DNA repair or chromosomal instability syndromes such as in xeroderma pigmentosum (Cleaver & Bootsma, 1975), ataxia telangiectasia (Taylor *et al.*, 1975), Fanconi's anaemia (Sasaki & Tonomura, 1973) or Bloom's syndrome (German, Bloom & Passarge, 1979), cells have been shown to be easily damaged by some carcinogens and the damage is not fully repaired. In dominantly inherited diseases with high cancer risks, however, a biochemical abnormality has not been detected, and chromosomal abnormalities in specific sites seem to play an important part in the carcinogenesis (Sasaki *et al.*, 1980).

Reed and Leone (1970) suggested that there were several populations (clones) of cells within the epidermis of PM, and these cells were more UV-sensitive than the normal epidermal cells. Cytogenetic studies of PM cells have been reported in only four cases (Taylor, Harnden & Fairburn, 1973): eight of nineteen fibroblast strains derived from the PM-affected area showed chromosomal instabilities, whereas unstable rearrangements, gaps or breaks were not increased. Chromosomal abnormalities were found in PM lesions associated with or without squamous cell carcinoma of the skin, but were not found in the unaffected areas.

Cultured fibroblasts in the present patient showed a shorter lifespan with chromosomal abnormalities, but there was no evidence of decreased UDS or CFA when compared with two other strains of PM, DSAP or with normal controls. The chromosomal abnormalities were mostly those of translocations and the sites were random, as in previously reported cases (Taylor *et al.*, 1973), but they were also found in cells from the unaffected area. Taylor *et al.* (1973) mentioned that interaction between dermal and epidermal cells should be studied before the chromosomal abnormalities were considered responsible for the development of skin cancers. In reviewing cytogenetic studies on dominantly inherited disorders with high cancer risks, Sasaki & Ejima (1981) stated that a mutation was dominantly expressed to initiate a neoplasm in a particular organ at a particular stage of development because certain types of procancer genes might express a mutator function and promote chromosome recombination in their somatic cells.

There has been no report on premature ageing in PM. Short stature, prematurely greying hair, diabetes mellitus and atrophic testes as well as shorter lifespan of the cultured fibroblast suggested a *forme fruste* of Werner's syndrome in the present patient. Age-dependent chromosomal rearrangements in fibroblasts and lymphocytes have been reported in Werner's syndrome (Scappaticci, Cerimele & Fraccaro, 1982). However, Werner's syndrome appears to be inherited by an autosomal recessive trait (Goto *et al.*, 1981), and the risk of developing malignancies, which are mostly mesenchymal, has been estimated to be less than 10% (Salk, 1982).

It is difficult to be certain whether our patient had a *forme fruste* of Werner's syndrome in addition to PM. The effects of other environmental carcinogens or of X-rays on the repair characteristics of the PM cells, as well as a study of fundamental mechanisms of the shorter lifespan of the cells, should be clarified before such a conclusion can be reached.

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Oral lichen planus: an immunoperoxidase study using monoclonal antibodies to lymphocyte subsets

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SUMMARY

Sixteen biopsies from patients with oral lichen planus (10), simple keratosis (3) and lichenoid reactions (3) were studied using monoclonal antibodies directed against lymphocyte markers. T lymphocytes predominated in the cellular infiltrates of the epithelium and lamina propria of all biopsies, and cells positive with Leu 3a and Leu 2a antibodies were also present at both sites in all specimens. However, many of the Leu 3a positive intraepithelial cells had a dendritic appearance and distribution consistent with their being Langerhans cells. Limited results from cell counts on three lichen planus specimens suggested that the majority of intraepithelial T cells were of the cytotoxic/suppressor phenotype (OKT3 and Leu 2a positive).

Lichen planus is a relatively common disorder affecting the skin and/or the oral mucosa (Andreasen, 1968a). Histologically, lesions of lichen planus are characterized by hyperkeratosis, liquefaction degeneration of the basal cell layer and the presence of a pronounced band-like subepithelial lymphoid infiltrate (Lever & Schaumberg-Lever, 1975; Andreasen, 1968b). The proximity of this infiltrate to the damaged epithelium has been used as evidence that immune mechanisms, particularly delayed hypersensitivity, are important in the pathogenesis of lichen planus.

A variety of studies, using conventional antibodies to B and T cell antigens, red cell adhesion tests and enzyme histochemistry, have consistently demonstrated the predominantly T-cell nature of the infiltrate in both cutaneous and oral lichen planus (Konttinen *et al.*, 1982; Braathen, Dal & Mellbye, 1979; Schmitt, Alario & Thivolet, 1979). However, recent attempts to investigate the *in situ* distribution of T-cell subsets in lichen planus lesions using monoclonal antibodies have yielded variable and contradictory results (Bhan *et al.*, 1981; McMillan *et al.*, 1981; Bjerke, 1982; Becker *et al.*, 1983; Willemze *et al.*, 1983) which together with the lack of

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simultaneous analysis of control tissues, do not allow further clarification of the possible role of immunological reactions in the aetiology and pathogenesis of the disease.

This paper investigates the cellular distribution of lymphocyte markers in three oral disorders, lichen planus, simple keratosis (leukoplakia) and lichenoid reactions provoked by non-steroidal anti-inflammatory drugs (Hamburger & Potts, 1983) using monoclonal antibodies to a variety of B- and T-cell markers.

METHODS

Tissue specimens

Biopsy specimens were taken from non-ulcerated mucosal lesions under local anaesthesia in patients with a clinical diagnosis of lichen planus, lichenoid drug reaction provoked by non-steroidal anti-inflammatory drugs and leukoplakia. The tissue was immediately bisected and one half placed in neutral buffered formalin for routine histological examination and the other half snap-frozen and stored in liquid nitrogen. Further study was confined to those frozen specimens whose routine histology was consistent with lichen planus (ten cases), lichenoid drug reaction (three cases) or simple keratosis (three cases).

Monoclonal antibodies

Table 1 lists the monoclonal antibodies used together with their specificity, source and dilution used in immunoperoxidase staining.

All antisera were evaluated on frozen sections of palatine tonsil. Staining patterns observed with antibodies to IgM, kappa, lambda, Leu 2a, Leu 3a and OKT3 were similar to those reported in the literature (Rouse *et al.*, 1982). Monoclonal antibody A50, reactive with 80% of peripheral blood T cells (Boumsell *et al.*, 1980), gave a similar staining pattern to that observed for OKT3 although the reaction product was less intense. D66, an antibody reactive with all circulating T cells, reacts most strongly with that population of cells forming E-rosettes at 37°C (Bernard *et al.*, 1982). This antiserum, used as an unfractionated ascites fluid, gave only weak staining of a few scattered lymphocytes in tissue sections but produced intense staining of vascular smooth muscle.

Immunoperoxidase method

Serial cryostat sections (5 μm) were mounted on gelatin-coated slides, usually four adjacent serial sections per slide, and air dried for 30 minutes at room temperature. The mounted

TABLE 1. Monoclonal antibodies against lymphocyte markers

Antibody	Specificity	Source	Dilution
OKT3	Pan T	Ortho	1:10
Leu 2a	Suppressor/cytotoxic T cells	Becton-Dickinson	1:40
Leu 3a	Helper/inducer T cells	Becton-Dickinson	1:40
A50	Pan T	Gibco	1:5
D66	E-rosette receptor/activated T cells	Gibco	1:5
FC3	Kappa chains	Seward	1:500
C4	Lambda chains	Seward	1:500
AF6	IgM	Seward	1:800

sections were then freeze dried for 4 hours, fixed in acetone (20 minutes at room temperature) and air dried for 5 minutes prior to immunostaining. Appropriate dilutions of the monoclonal antibodies were applied directly to the air-dried sections which were incubated for 60 minutes at room temperature. After washing in 0·05 M Tris-HCl buffered saline pH 7·6 (TBS) (3 × 2 minutes) sections were overlaid with peroxidase conjugated rabbit anti-mouse Ig (Dakopatts As., Denmark; 1:50 dilution) for 30 minutes at room temperature. Unbound conjugate was removed by washing in TBS (3 × 2 minutes) and bound peroxidase visualized using diaminobenzidine reagent. All sections were counterstained with Mayer's haematoxylin prior to dehydration and mounting in Canada balsam. TBS was used for washing and reagent dilution.

Controls included omission of primary and secondary layers and replacement of the primary layer with normal mouse serum.

Evaluation of sections

Estimates of the number of positive cells within the infiltrate were made for each monoclonal antibody as indicated in Table 2. For adjacent serial sections stained with OKT3, Leu 2a and Leu 3a, the total number of positive cells was counted throughout the whole of the epithelium. The number of positive cells in the lamina propria was determined in five high-powered fields randomly distributed immediately beneath the basement membrane in sections of lichen planus and lichenoid reactions. In simple keratosis, where only small numbers of lymphocytes were present, the positive cells were counted in five high-powered fields centred on foci of lymphocytes in the lamina propria adjacent to the basement membrane.

RESULTS

The results are summarized in Table 2, which indicates that T cells predominate in the lymphoid infiltrate of the epithelium and lamina propria in all the biopsies. B cells were never detected within the epithelium and only small numbers of B cells were detected in the lamina propria of four specimens (three lichen planus and one lichenoid reaction). Of the two monoclonal antibodies reactive with all circulating T cells, OKT3 consistently gave a stronger reaction product and stained more cells than A50. The E-rosette receptor antibody (D66) stained small numbers of lymphocytes in two specimens, but this antibody stained smooth muscle surrounding blood vessels in all biopsies (Fig. 1).

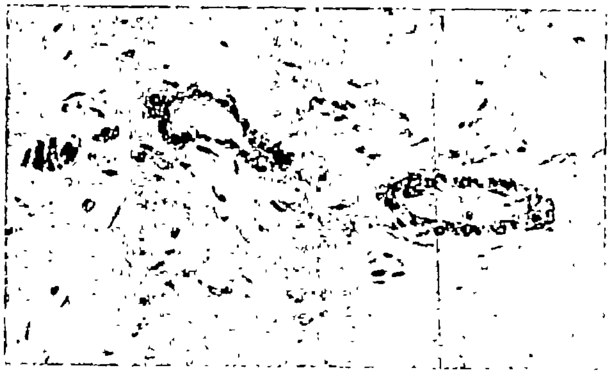

FIGURE 1. Vascular smooth muscle stained with monoclonal antibody D66 (× 160).

TABLE 2. Summary of staining results[a]

	Epithelium								Lamina propria							
	B-cell markers			T-cell markers					B-cell markers			T-cell markers				
Diagnosis	IgM	κ	λ	OKT3	A50	Leu 2a	Leu 3a	D66	IgM	κ	λ	OKT3	A50	Leu 2a	Leu 3a	D66[b]
Lichen planus	–	–	–	++	++	++	+++[c]	(+)	(+)	(+)	(+)	+++	++	++	+++	(+)
Lichenoid drug reaction	–	–	–	++	++	++	+++[c]	–	(+)	–	–	+++	++	++	+++	–
Keratosis	–	–	–	++	++	++	+++[c]	–	–	–	–	+++	++	++	+++	–

[a] Staining assessed on a scale (–) none, (+) 1–20%; (++) 21–50%; (+++) > 50% cells in the infiltrate positive

[b] Intense staining of vascular smooth muscle in all biopsies. Results in brackets indicate that not all specimens contained positive cells.

[c] Majority of positive cells were dendritic.

TABLE 3. Leu 3a$^+$: Leu 2a$^+$ cell ratios in biopsy specimens

	Leu 3a$^+$: Leu 2a$^+$ cell ratio				
	Epithelium		Lamina propria		
Diagnosis	Mean	Range	Mean	Range	Number of cases
Lichen planus	2·1:1	1·5–3·2:1	2·5:1	1·6–4·1:1	10
Lichenoid drug reaction	1·8:1	1·4–2·2:1	2·0:1	1·6–2·4:1	3
Keratosis	2·6:1	2·0–3·1:1	2·7:1	1·6–3·3:1	3

Cells positive with Leu 3a and Leu 2a antibodies were present in all specimens (Fig. 2; Table 2) and cell counts demonstrated mean Leu 3a : Leu 2a positive cell ratios of 2·1 : 1 and 2·5 : 1 in epithelium and lamina propria respectively for lichen planus specimens (Table 3). Similar ratios were obtained for the control tissues of lichenoid reactions and simple keratosis.

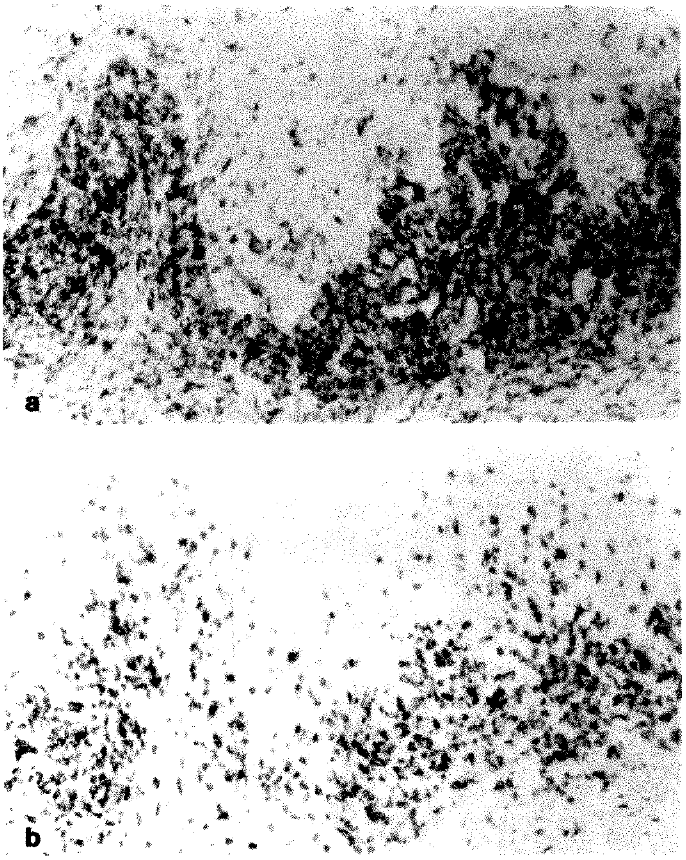

FIGURE 2. Lichen planus specimen stained with Leu 3a (a) and Leu 2a (b) showing the predominance of Leu 3a positive cells within the epithelium and lamina propria (× 100).

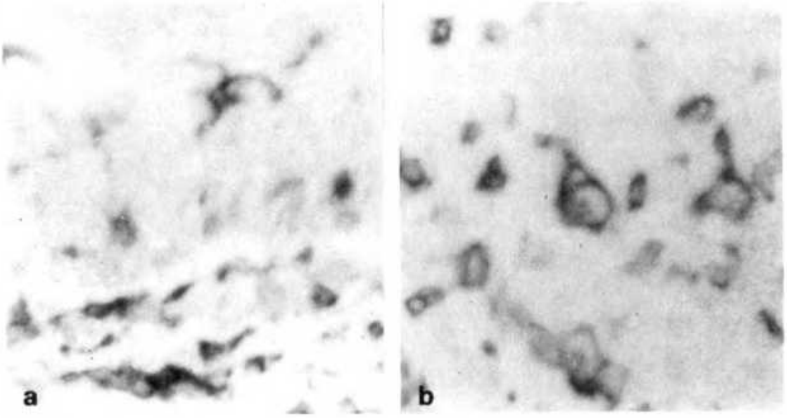

FIGURE 3. Dendritic (a) and large mononuclear (b) Leu 3a positive cells within the epithelium (× 400).

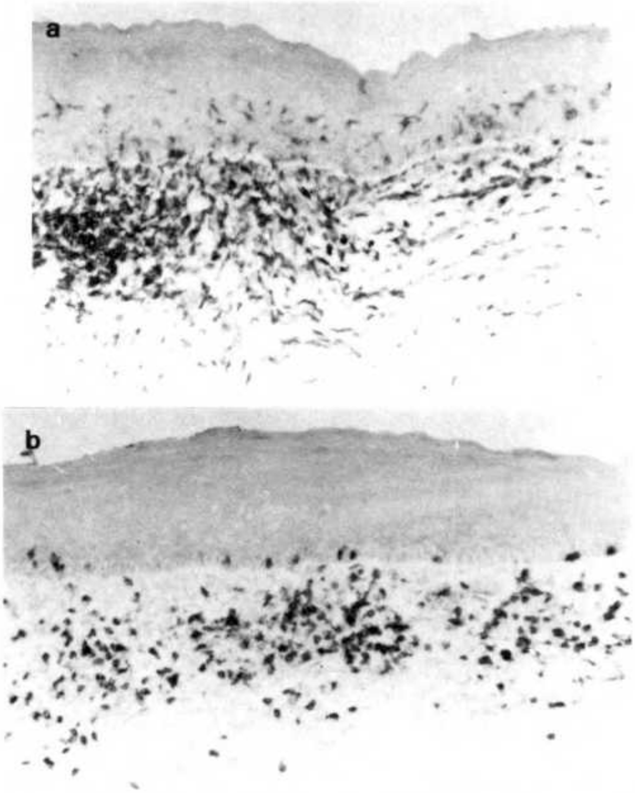

FIGURE 4. Lichen planus specimen stained with Leu 3a (a) and Leu 2a (b) illustrating the basal cell location of Leu 2a positive cells found in many biopsies (× 100).

TABLE 4. Lichen planus: intraepithelial cell counts

Case	OKT3	Leu 2a	Leu 3a
1	97	116	392
2	317	382	630
3	96	110	261

[a] Total number of positive intraepithelial cells per section. Adjacent serial sections counted for each case.

Many of the intraepithelial Leu 3a positive cells were concentrated in the stratum spinosum and had a dendritic appearance (Fig. 3). In contrast OKT3 and Leu 2a positive intraepithelial cells were usually found in the basal cell layer and the lower stratum spinosum (Fig. 4). In all specimens the number of intraepithelial Leu 3a positive cells was always in excess of those positive with other T-cell markers, including the two pan T-cell antibodies (OKT3, A50). In the three cases of lichen planus where adjacent serial sections had been stained for OKT3, Leu 2a and Leu 3a, and comparable cell counts for all these markers could be made, it was clear that the total number of Leu 2a and Leu 3a positive cells exceeded the number of OKT3 positive cells while equivalent numbers of OKT3 and Leu 2a positive cells were present within the epithelium (Table 4).

DISCUSSION

Our results confirm the preponderance of T cells in the mucosal lymphoid infiltrates of oral lichen planus and extend this observation to include lichenoid drug reactions and the sparse infiltrates in oral keratosis. All specimens contained a mixed infiltrate of Leu 3a positive and Leu 2a positive cells within the lamina propria. The ratio of Leu 3a:Leu 2a positive cells of approximately 2:1 in the oral lichen planus specimens agrees with two reports of skin lesions documenting seven cases (Bhan *et al.*, 1981; Bjerke, 1982). A third study (McMillan *et al.*, 1981) demonstrated only occasional OKT8 positive and no Leu 2a positive cells in the infiltrate of one case of cutaneous lichen planus. However, cryostat sections fixed in paraformaldehyde were used, and the authors indicate that technical difficulties may have been responsible for these results. Willemze *et al.* (1983) in a study of the T-cell subsets in cutaneous lymphoma include one case of lichen planus amongst their controls. This one case showed a Leu 3a:Leu 2a ratio of 15:1 in the dermal infiltrate. Technical problems are unlikely to underlie this unusual result. More surprising is the difference between our results and those reported for skin lesions with the findings of Becker *et al.* (1983) who found a preponderance of OKT8 positive cells in the lymphoid infiltrates of both epithelium and lamina propria in twenty-three lesions of oral lichen planus using an immunofluorescence method. Although this latter study was performed with different monoclonal antibodies to the T-cell subsets (OKT) this is unlikely to account for the difference. It is accepted that Leu 3a and OKT4 antibodies are reactive against the same antigen, which is expressed by helper/inducer cells. Similarly Leu 2a and OKT8 antibodies detect another antigen expressed by the cytotoxic/suppressor subset of T cells.

Only two of the previously reported studies have commented on the presence of positive intraepithelial cells. Bjerke (1982) found approximately equal numbers of OKT4 and OKT8 positive cells in the epidermis whereas Becker *et al.* (1983) demonstrated a preponderance of OKT8 positive cells in the epithelial infiltrates of oral lesions. Neither study detected positive intraepithelial dendritic cells with these antibodies. The dendritic nature of many of the Leu 3a positive intra-epithelial cells in our study was of interest because of their similarity to Langerhans cells, which have been shown to express this antigen in some conditions (Wood, Warner & Warnke, 1983). It is unlikely that the staining of Langerhans cells is produced by binding of the second layer peroxidase-conjugated rabbit antibody to Fc receptors on Langerhans cells, as they were not detected when the primary antibody was omitted or when monoclonal antibodies to other antigens were used. Our results thus appear to confirm those of Wood and co-workers and suggest that the majority of intraepithelial Leu 3a positive cells detected are Langerhans cells and that the majority of intraepithelial T cells in oral lichen planus are of the suppressor/cytotoxic phenotype (OKT3, A50, Leu 2a positive). This conclusion is in general agreement with the distribution of intraepithelial T-cell subsets reported by Becker *et al.* (1983).

The work of Wood *et al.* (1983) also suggested that some macrophages, in addition to Langerhans cells, expressed the Leu 3a/OKT4 antigen. It is interesting to note that large numbers of macrophages are present in the inflammatory cell infiltrate within the lamina propria of oral lichen planus lesions (Kontinnen *et al.*, 1982; Becker *et al.*, 1983). We have also demonstrated lysozyme-positive mononuclear cells both within and beneath the epithelium of such lesions using paraffin sections (unpublished observations). It is therefore important to recognize that some of the Leu 3a positive cells found in the lamina propria in our and other reported studies may be macrophages rather than T cells. Further work is required to test this possibility, which may again explain some of the differences between our results and those of Becker *et al.* (1983). Although the staining reaction of Langerhans cells and macrophages was weak and apparently cytoplasmic it is difficult to understand why these and previous workers have not detected similar reactivity with Leu 3a and OKT4 monoclonal antibodies.

In conclusion, our preliminary studies indicate that the overall staining patterns observed with monoclonal antibodies to the lymphocyte markers were similar in all lesions of oral lichen planus, lichenoid drug reactions and simple keratosis. However, the predominance of intraepithelial T cells expressing Leu 2a reactivity and their distribution within the basal cell layer close to the area of cell damage in lichen planus supports the view that cytotoxic T cells have a role in the pathogenesis of this condition, as has been suggested in the lichen planus-like skin lesions seen in graft-versus-host disease (Lampert *et al.*, 1981). More extensive studies on a larger series of cases are in progress using monoclonal antibodies to macrophage, Langerhans cell and lymphocyte markers to confirm these observations and obtain more information on the lymphoid infiltrates of all three conditions.

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Low serum zinc in children with atopic eczema

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SUMMARY

Serum zinc was measured by atomic absorption spectrophotometry in sixty-five children with atopic eczema and seventy-nine control children. The mean serum zinc of the patients, ($11{\cdot}4 \pm 2{\cdot}0$ μmol/l) was significantly lower than that of the controls ($13{\cdot}7 \pm 2{\cdot}3$ μmol/l, $P < 0{\cdot}0001$). There was no significant correlation between the patients' serum zinc concentration and either the height/weight centile or a subjective assessment of severity and extent of the eczema. Of eleven patients with serum zinc below 10 μmol/l, six had recurrent infections of the skin, a significantly greater proportion than in patients whose serum zinc was 10 μmol/l or above. It is suspected that the decreased plasma zinc concentration in children with eczema is a non-specific consequence of the dermatological disorder, and therefore there is no indication for zinc supplementation.

The skin lesions of acute zinc deficiency have some eczematous features (Weismann, 1980). It also appears that significant losses of zinc can occur through desquamation (McMillan & Rowe, 1983; Molin & Wester, 1973). We here report an hitherto undescribed low serum zinc level in children with atopic eczema.

METHODS

Sixty-five children with atopic eczema, referred because of poor response to conventional topical treatment, were investigated for possible causative factors such as allergy, immune deficiency and zinc deficiency. No patients were receiving systemic corticosteroids. Severity of eczema was crudely assessed as mild, moderate or severe, and extent assessed as mild (less than 10% surface area affected), moderate (10–50% surface affected) or severe (over 50% surface affected). No patients had a skin infection at the time of sampling.

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Venepuncture was carried out between 11.00 and 12.00 hours avoiding the effects of diurnal variation of the serum zinc (Lifschitz & Henkin, 1971; McMillan & Rowe, 1982), and an aliquot for serum zinc assay was allowed to clot in a plastic tube. After centrifugation the separated serum was stored in plastic tubes at 4°C prior to analysis. Serum was employed despite an earlier report of increased zinc content of serum compared with plasma (Foley *et al.*, 1968), as recent evidence indicates no significant difference (Kiilerich *et al.*, 1981). Analysis of zinc was by atomic absorption spectrophotometry (Pye SP192). Aqueous standards, controls and tests were diluted one in ten in de-ionised water and analysed in duplicate, with background correction. The standard deviation, between batches, at a level of 9·0 μmol/l was ±0·34 μmol/l. The seventy-nine controls were patients who were having blood taken for blood grouping prior to tonsillectomy or elective general paediatric surgery.

RESULTS

Both groups contain patients from about 2 months of age to 16 years. Whilst the age distribution is fairly uniform in the control group, it is skewed towards the younger ages in the eczema group (57% of the cases being under 3) (Fig. 1). There were roughly twice as many males as females in both the patient and the control group.

There was no evidence for any difference between median zinc concentration in males and females in either group, so results for both sexes were pooled together. Zinc concentrations in controls were significantly negatively correlated with age ($r = -0{\cdot}314$, $P = 0{\cdot}002$). Examination of the scattergram of zinc concentration with age indicated high levels in controls aged under 3 years. A Mann–Whitney U test comparing controls aged under 3 years with those 3 years old and over gave a significant result ($P = 0{\cdot}0001$). For patients there was no evidence that zinc concentration and age were related.

The mean serum zinc concentration of the patients, 11·4 ± 2·0 (median 11·3) μmol/l, was significantly lower than that of the controls, 13·7 ± 2·3 (median 13·4) μmol/l, Mann–Whitney $U = 160$, $P < 0{\cdot}0001$. Because of the curiously high zinc concentrations in controls under 3, the test was repeated with controls and patients under 3 years excluded. Again a similar highly significant difference was found between patients (mean zinc 11·5 μmol/l, median 11·5, $n = 28$) and controls (mean zinc 13·2 μmol/l, median 12·9, $n = 66$), Mann–Whitney $U = 482$, $P = 0{\cdot}0002$.

A Kruskal-Wallis analysis of variance showed no significant difference in serum zinc for the different levels of severity or extent of the eczema. There was no significant correlation between height and weight centiles and serum zinc concentration. Taking the eleven patients with a low serum zinc concentration (below 10 μmol/l), there was again no significant correlation with height or weight centiles.

On re-examination of the records of the eleven patients with serum zinc below 10 μmol/l, it was noted that two had autosomal dominant ichthyosis. In six of the eleven (54%) recurrent infection of the skin was a prominent feature, compared with eleven of the other fifty-four (20%) ($P = 0{\cdot}03$, Fisher's exact test).

DISCUSSION

Previous studies of normal newborns and infants have shown that a fall in the serum zinc concentration to adult levels is complete by 3 months of age (Berfenstam, 1952; Henkin *et al.*, 1973; Gibson & Dewolfe, 1981). The significantly higher serum zinc concentrations in controls under 3 years is thought to be spurious and due to sampling error (small numbers) and one very

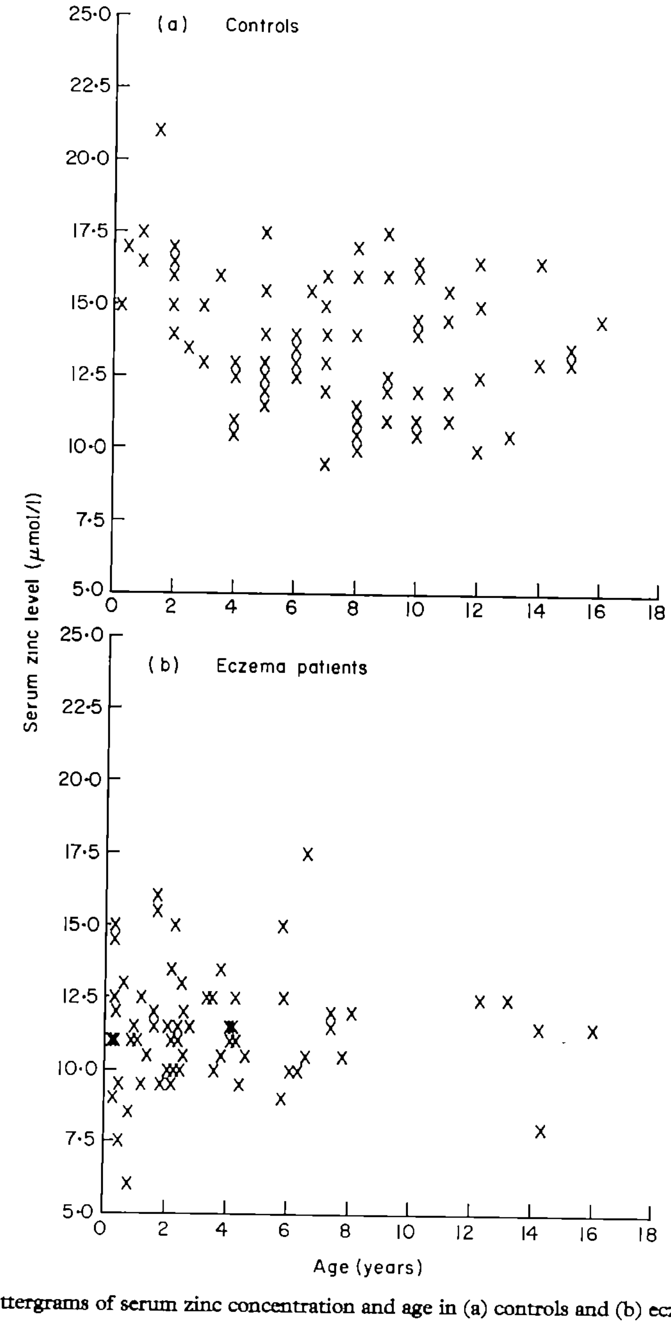

FIGURE 1. Scattergrams of serum zinc concentration and age in (a) controls and (b) eczema patients.

high result. This artefact complicated the results by slightly exaggerating the significance of the low serum zinc of the patients under 3 years of age. However, even when these younger patients were excluded, the low serum zinc in patients with atopic eczema remained significantly low. The differing age distributions of the two groups does not explain the finding of a low serum zinc. There is a linear relationship between albumin concentration and zinc concentration in the blood, and circulating zinc is in part bound to albumin (Solomons, 1979). Albumin was not measured in this study, and it is theoretically possible that a difference in albumin levels in the two groups may be an explanation of the results.

Unfortunately, for a variety of reasons (Solomons, 1979), a plasma or serum zinc concentration alone is unlikely to reflect reliably total body zinc status. The circulating zinc concentration can provide only a portion of the information necessary to assess the zinc status of an individual. The reasons for a low serum zinc concentration in atopic eczema are obscure. Were loss of zinc in the skin to be the explanation there should be a significant correlation between serum zinc and an assessment of extent and severity of disease, but there was not. The rather crude subdivision of extent and severity is not likely to have obscured a correlation with the serum zinc.

It appears that some children with eczema have short stature (unpublished observations), for reasons that are obscure. Zinc deficiency is known to cause growth failure (Prasad, 1982; Hambidge, 1981), and low serum zinc concentrations are found in certain diseases in which growth failure is a feature, such as cystic fibrosis (Halsted & Smith, 1970; Neve *et al.*, 1983; Hansen *et al.*, 1983) and coeliac disease (Naveh *et al.*, 1983). The absence of a significant correlation between the patients' serum zinc concentrations and their height or weight centiles argues against this association being an explanation for our results.

A decreased plasma zinc concentration has been reported in a small group of patients with ichthyosis (Greaves & Boyde, 1967), the autosomal dominant form of which is common in children with atopic eczema. But of the eleven patients with serum zinc concentration below 10 μmol/l, only two had ichthyosis. However, these eleven patients had a significant excess of those with recurrent infection of the skin. Depression of plasma zinc has been noted in both animal (Beisel, 1977) and human infections (Beisel, 1977; Gordon, Gordon & Passal, 1981), partly due to re-distribution with an accelerated flux of zinc from the serum into the liver (Beisel, 1977). Zinc supplementation does not improve the response to infections in animals (Beisel, 1977).

The absence of significant correlation between serum zinc concentration and subjective asessment of severity or extent of eczema, or height or weight, suggests that the decreased plasma zinc concentration in children with atopic eczema is a non-specific finding, akin to the non-specific lowering of serum iron concentration in patients with skin disease (Shuster, 1967). Low serum zinc concentrations have also been reported in a number of other dermatological conditions (Greaves & Boyde, 1967). Despite finding a low serum zinc in children with atopic eczema this does not appear to represent zinc deficiency, and therefore there is no indication for zinc supplementation in these patients.

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Pharmacology and Treatment

The response of seborrhoeic dermatitis to ketoconazole

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SUMMARY

A randomized double-blind placebo-controlled cross-over study was made of ketoconazole 200 mg daily in nineteen patients with seborrhoeic dermatitis. All had scalp lesions and sixteen had seborrhoeic dermatitis at other sites. Responses were measured by clinician and patients independently, using a linear analogue scale. Body and scalp lesions and itch regressed considerably and significantly with ketoconazole in all but five patients, three of whom subsequently responded to a higher dose. The patients studied with seborrhoeic dermatitis had been sent by their family doctors in answer to a request for patients with dandruff, and the clinical difference between the two was found to be only of degree. Three patients with dandruf without erythema were studied separately using the same study design: all three responded similarly to those with seborrhoeic eczema. It is concluded that Pityrosporum yeast infection is the immediate cause of seborrhoeic dermatitis and that dandruff is its mildest manifestation.

Seborrhoeic dermatitis is common and easily recognized but its cause is unknown. Despite its name no quantitative or qualitative abnormality of sebum production has been found (Pye, Meyrick & Burton, 1977; Burton & Pye, 1983). Although an aetiological role for the Pityrosporum yeasts was proposed long ago (Sabouraud, 1904), most now consider the increased numbers of these micro-organisms found in seborrhoeic dermatitis of the scalp to be a secondary colonization of the rash (Leyden, McGinlay & Kiigman, 1976; Kligman & Leyden, 1983). The availability of ketoconazole, an orally administered imidazole derivative which is active against the Pityrosporum yeasts both *in vitro* (Van Cutsem, 1983) and *in vivo* (Borelli, 1980; Ford, Ive & Midgley, 1982), allowed us to put these contrary views to the test. We report the results of a double-blind randomized controlled trial of ketoconazole in seborrhoeic dermatitis.

METHODS

Nineteen subjects (ten male, nine females, aged 18–60 years) with unequivocal seborrhoeic dermatitis were studied during the winter months. All had scalp lesions and sixteen had seborrhoeic dermatitis at other sites. None were taking oral medication apart from those females who were taking an oral contraceptive.

The patients entered a double-blind, randomized, controlled, cross-over study which lasted for 12 weeks. During this time they were asked to wash their hair twice weekly with 10 ml of 10% Green Soft Soap Solution BP. No other scalp preparation was used and the patients were requested not to wash their hair for 3 days before each assessment.

For the first 4 weeks of the study the subjects received no medication. Each subject then received either ketoconazole 200 mg per day for 4 weeks, or placebo of identical appearance, allocated randomly. After 4 weeks, those receiving ketoconazole were changed on to placebo and vice-versa.

The subjects were examined by the same two investigators at 4 weekly intervals, when a clinical assessment on a 0–4 scale was made of scalp scaling and seborrhoeic dermatitis at other body sites. Because of the difficulty in assessing scalp erythema it was attempted only when the

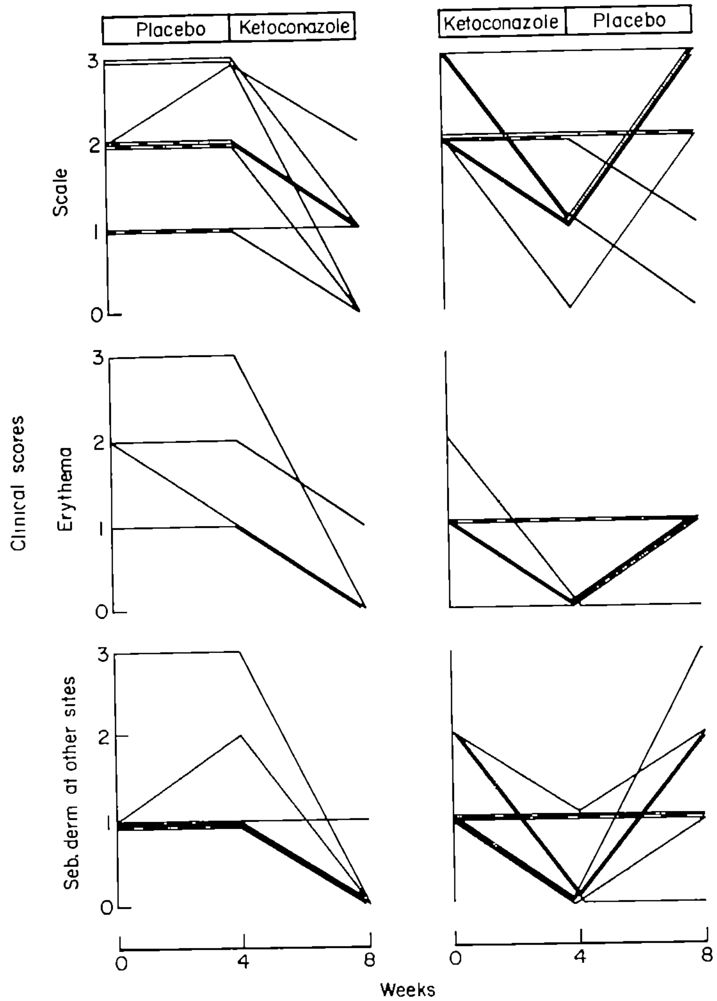

FIGURE 1. Clinical assessment gradings for individual patients.

whole scalp was involved. In addition, each subject made his or her own weekly assessment of scalp scaling and itching, on a 100 mm analogue scale, the extremes of the scale being 'none' and 'severe' for each variable.

After completion of the study, four of the five subjects who had failed to respond to 200 mg ketoconazole per day were given 400 mg per day for 4 weeks in an open manner. The three patients with dandruff had scaling without erythema and differed only in degree from the patients with seborrhoeic dermatitis. They were treated with 200 mg ketoconazole daily using the same double-blind placebo-controlled cross-over regimen.

The Wilcoxon signed rank test was used to analyse the clinical grading. The linear scales were analysed using the two-tailed *t*-test.

RESULTS

Two subjects were withdrawn from the study: one developed a transient widespread erythematous rash after 7 days of treatment with ketoconazole and the other moved from the area during the period of the study.

Analysis of the clinical assessment gradings (Fig. 1) showed that for scalp scaling, twelve (70%) of the seventeen subjects completing the study showed considerable improvement on

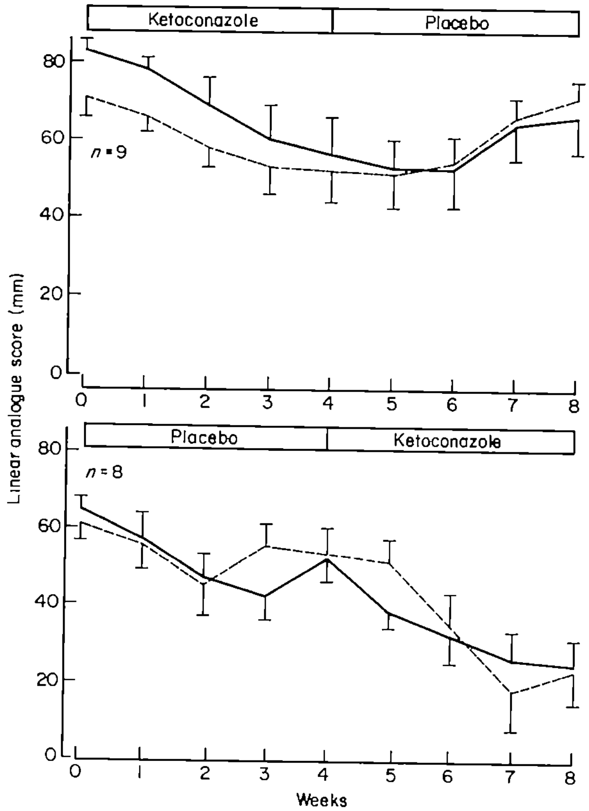

FIGURE 2. Mean (± s.e.m.) score on linear analogue scale for itch (– – –) and scale (——) in each group of patients.

ketoconazole whereas only two subjects improved with placebo. This difference in response was significant ($P < 0{\cdot}01$). Scalp erythema was graded only in the ten subjects in whom it was generalized. The improvement in gradings was significantly greater ($P < 0{\cdot}05$) for ketoconazole compared with placebo. Of the fifteen subjects with seborrhoeic dermatitis at other sites in addition to the scalp, total clearance was noted in ten whilst on ketoconazole and analysis of the changes in severity of grading revealed them to be significant ($P < 0{\cdot}01$). There was a significant reduction ($P < 0{\cdot}01$) in linear analogue assessments of scalp scaling and itch by the 4th week of ketoconazole treatment (Fig. 2).

When the group that received ketoconazole first was changed over to placebo, relapse occurred for most subjects by 4 weeks, one subject remaining free of symptoms for a further 2 weeks. In the group that received placebo first, a response was recorded for both scale ($P < 0{\cdot}02$ at week 3) and itch (not significant) for the first 2 to 3 weeks but by the time of cross-over to ketoconazole this improvement was no longer apparent. Four of the five subjects who did not respond to the 200 mg ketoconazole a day used in the study were subsequently given 400 mg per day for 4 weeks in an open study: three had a considerable objective and subjective improvement of their seborrhoeic dermatitis while taking the higher dose. In all three patients with dandruff, the scale and itch (linear analogue) likewise responded greatly to 200 mg ketoconazole daily.

DISCUSSION

The present study has demonstrated a considerable effect of ketoconazole on seborrhoeic dermatitis of the scalp and other sites. Although we used subjective methods for clinical grading of scalp scaling and erythema and seborrhoeic dermatitis at sites other than the scalp, the double-blind, randomized controlled, cross-over design allows reasonable confidence in the results. The changes in scalp scaling, scalp erythema and seborrhoeic dermatitis at other sites in response to oral ketoconazole were all clinically obvious, and were mirrored by the improvement of scalp scaling and itch which the patients recorded on linear analogue scales. The responses were significantly greater than the small response to placebo. Because we had no objective measurements of response, we do not know whether this small placebo effect was on the disease or on its assessment. In keeping with these findings three of four non-responders subsequently responded to an increased dose of ketoconazole. The rapid response of seborrhoeic dermatitis to the pityrosporicidal agent ketoconazole clearly suggests that pityrosporal infection is primary and not, as currently believed, secondary. The reason only certain individuals appear prone to develop a Pityrosporum infection and how this leads to a dermatitis now require investigation. Clemmenson and Hjorth (1983) have shown ketoconazole to be similarly effective in a group of patients with head and neck dermatitis with positive prick tests to *Pityrosporum orbiculare*. Some of these patients had atopic eczema and no doubt others had what we would have regarded as seborrhoeic dermatitis.

Our study appears also to throw light on the relationship of dandruff to seborrhoeic dermatitis. The subjects included in this study were collected following a request to general practitioners to refer patients with troublesome dandruff. In the event we found that most of the referrals, nineteen patients, had seborrhoeic dermatitis of the scalp and sixteen of these patients had seborrhoeic dermatitis on other parts of the body. Only three referrals had chronic diffuse and excessive scaling of the scalp without evidence of inflammation, i.e. dandruff, and a few had scalp psoriasis, lichen simplex or atopic eczema. It is clear therefore that most patients who were considered by themselves and their family doctors to have dandruff did in fact have seborrhoeic dermatitis. Although the three patients with scaling without erythema appeared to differ only in degree and were not included in the controlled study, in all three the dandruff cleared and the subjects' linear analogue assessments of scalp scaling and itch also improved considerably whilst taking 200 mg ketoconazole daily. We therefore conclude that in most patients complaining of dandruff the condition is indistinguishable from mild seborrhoeic dermatitis and responds to ketoconazole in the same way. As with seborrhoeic dermatitis, the nature of the association between dandruff and the Pityrosporum yeasts has been a point of controversy for many years. The present findings therefore give strong support to the view that infection by these yeasts is the primary cause of dandruff (Shuster, 1984).

The present study was done to provide aetiological evidence and not to establish a practical therapy. Since seborrhoeic dermatitis rapidly relapses when ketoconazole is stopped and since ketoconazole may occasionally produce hepatotoxicity (Peterson, David & Kirkpatrick, 1980; Heiberg & Svejgaard, 1981; Janssen & Symoens, 1983) and interferes with testosterone metabolism (Schurmeyer & Nieschlag, 1982), the drug is not suitable for prolonged treatment of seborhoeic dermatitis and dandruff, its minor manifestation. It is to be hoped that an equally effective topical preparation or derivative will be developed.

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Low-dose oral chloroquine in the treatment of porphyria cutanea tarda

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SUMMARY

Seven patients with porphyria cutanea tarda received a total of ten courses of low-dose oral chloroquine therapy (125 mg chloroquine phosphate twice weekly). Patients were treated for a mean 14·9 months during which time all went into clinical and biochemical remission. Relapse occurred in four patients on a total of six occasions after a mean 17 months. In four patients there was no relapse after a mean 47·3 months. There were no adverse side-effects from the treatment. Low-dose oral chloroquine therapy appears to be a safe, effective and convenient treatment for porphyria cutanea tarda, although relapses may occur requiring further therapy.

Synthetic agents introduced for the treatment of malaria shortly before the Second World War were also found to have some efficacy in patients with discoid lupus erythematosus (Popoff & Kutintscheff, 1943), rheumatoid arthritis (Page, 1951) and certain photosensitive states, especially polymorphic light eruption (Cahn *et al.*, 1954; Knox *et al.*, 1954). Some of these chloroquine-treated patients also suffered from porphyria cutanea tarda (PCT) and such people were noted on occasion to develop an exacerbation of the porphyrin metabolic defect in association with malaise, fever and signs of liver dysfunction (Teodorescu *et al.*, 1959; Embree, 1961; Sweeney *et al.*, 1962). In patients with discoid lupus erythematosus and PCT, Cripps and Curtis (1962) found an immediate marked increase in urinary uroporphyrin output following 1·0–2·5 g chloroquine taken orally over 2–6 days; the maximum urinary uroporphyrin in one patient reached 53 mg per day after 6 days' treatment (normal maximum urinary uroporphyrin 40 μg (49 nmol)/24 h). Excretion returned to and then fell below pretreatment levels within 2–3 months. Early temporary liver enzyme disturbance was also noted. Felsher and Redeker (1966) also found an early rise in urinary uroporphyrin levels in six PCT patients treated with antimalarials. In response to 2–11 days of oral chloroquine in a daily dose of 0·5–1·0 g, the urinary uroporphyrin values reached 3·6–10·8 mg per day after 4–5 days' treatment, but returned to and then fell below pretreatment levels within 2–5 months. An early brief abnormality in liver enzyme levels was again noted. More recently, similar results were again obtained when the same type of regime was used as a rapid treatment for PCT in unreliable attenders (Tsega *et al.*, 1981). On the other hand, in a successful attempt to treat PCT but at the same time to avoid the acute hepatotoxic effects of chloroquine, Taljaard *et al.* (1972) used a low-dose regime of 300 mg orally twice weekly over a longer period of time (2–16 weeks). They found only small increases in the already raised pretreatment 24 h urinary uroporphyrin concentrations, and these subsequently dropped below the pretreatment values to almost normal levels. Only one of the eight patients relapsed after completing treatment, although the length of follow-up was not mentioned. In a much larger series of patients, Malina & Chlumský (1973), Kordač & Semrádová (1974) and Kordač, Papežovà & Semrádová (1977) also used low-dose oral chloroquine as a treatment for PCT and reported satisfactory responses and long remissions for up to 4 years. They administered chloroquine 125 mg twice weekly for 8–18 months, and discontinued it when the urinary uroporphyrin concentration fell below 100 μg (83 nmol)/24 h. Virtually all of their 112 patients remained in remission for at least 21 months, and 73 for more than 3 years. On the other hand, more recently Malkinson and Levitt (1980), giving hydroxychloroquine, have found early relapses within 24 months in every one of four patients studied. These conflicting reports have led us to document our own experience of low-dose oral chloroquine therapy in PCT.

METHODS

Seven male patients with PCT attending the photobiology out-patient clinic at St John's Hospital for Diseases of the Skin, London, were treated with low-dose oral chloroquine therapy (Table 1). The patients' ages ranged from 37 to 70 years (mean 57 years). All had the clinical and biochemical abnormalities of PCT and were initially treated in hospital for about 2 weeks, after which they were followed up monthly as out-patients. Liver biopsy was carried out in each case before treatment commenced, and liver function was regularly checked thereafter, as were the concentrations of urinary porphyrin, serum iron and blood haemoglobin. Ophthalmological examinations to exclude chloroquine oculotoxicity were undertaken at the Institute of Ophthalmology, London, before therapy and every 6 months until therapy ceased. The dose of chloroquine was 125 mg twice weekly, except in one course of therapy for which it was 125 mg on alternate days, and treatment was continued in each case until urinary uroporphyrin excretion had fallen to or below about 100 nmol/24 h.

RESULTS

All seven patients went into clinical and biochemical remission on ten separate occasions (some relapsed and needed retreatment) after a mean 14·9 months (range 10–23 months). Urinary uroporphyrin levels prior to the courses of treatment were very variably raised to between 189 and 55,487 nmol/24 h (mean 7251 nmol/24 h). During the first few days of therapy, these values either fell gradually (in two out of ten courses of treatment in the seven patients), or rose briefly (in seven out of ten courses of treatment in the seven patients) to a maximum of 16,080 nmol/day to be followed by a steady decrease towards normal. The relapses occurred in four patients on a total of six occasions at a mean 17 months (range 3–35 months) after completion of treatment, three of the four relapsed patients being successfully retreated and two then relapsing again. The other relapsed patient was also retreated, the course of therapy being still in progress after only 3 months. One retreated patient and three single treatment patients remained in remission after a mean 47·3 months (range 20–59 months). Of the four patients who admitted to taking alcohol, one patient was still in remission after 51 months although he was still a heavy drinker;

TABLE 1. Clinical details of low-dose oral chloroquine therapy in 7 patients with porphyria cutanea tarda

Name	Age at start of treatment	Treatment starting date	Treatment duration (months)	UP			CP		AsT			AlT		Fe		Duration of remission after treatment stopped (months)	Date relapse noted (i.e. UP > 100)	Alcohol intake before and after diagnosis	Comments and pretreatment liver histology
				1	2	3	1	3	1	2	3	1	3	1	3				
G J	37	Sept '76	14	827	2891	65	213	80	67	69	26	76	21	37	50	35	Oct '80	High beer + spirits Stopped spirits	Poor attender Virtually normal liver
H O	51	Dec '75	13	2440	8333	265	635	121	100	126	26	132	26	49	24	13	Feb '78	Moderate beer + spirits Stopped spirits	Fibrosis of liver
		Mar '78	18	2099	ND	20	283	35	175	140	32	180	31	38	44	17	Feb '81		
J M	55	Aug '76	17	55,487	17,544	70	2545	96	37	118	13	24	7	24	18	25	Jan. '79	Minimal	Drug addict Cirrhosis
		Apr '80	11	2114	1227	48	761	292	19	15	19	17	10	17	ND	9	Dec '81		
R S	60	Dec '76	13	521	146	50	103	72	30	52	37	41	45	22	21	59	No relapse	Nil	Marked pre-cirrhotic liver changes
E.H	62	Jan '79	14	4200	16,080	134	2142	92	53	134	21	86	26	36	ND	3	June '80	Minimal beer intake.	Mild diabetic
		June '80	10	189	309	72	156	196	21	ND	17	26	24	ND	23	20	No relapse	Nil	Mild inflammatory and fatty liver changes
J B	65	Jan '77	16	282	207	76	108	48	35	34	25	51	28	33	31	55	No relapse	Nil	Progressive cirrhosis
N B	70	Jan '76	23	4351	8690	51	736	94	45	64	25	80	23	21	24	55	No relapse	High wine + spirit intake	Virtually normal liver
Mean	57		14·9													29·1			

All patients were male. Each was treated orally with chloroquine phosphate 125 mg twice weekly, except H O who was given 125 mg on alternate days from January 1976 to March 1976. Tests were carried out monthly

UP = urinary uroporphyrin (nmol/24 h) (normal 0–49)
CP = urinary coproporphyrin (nmol/24 h) (normal 15–432)
AsT = serum aspartate transaminase (IU/l) (normal 5–35)
AlT = serum alanine transaminase (IU/l) (normal 5–35)
Fe = serum iron (μmol/l) (normal 14–30).
ND = not done
1 = just prior to commencement of chloroquine therapy
2 = during chloroquine therapy (maximum measured level)
3 = at cessation of chloroquine therapy

the three others, who also continued their drinking, were amongst those who relapsed. The serum liver transaminase enzymes showed increased pretreatment activity in five of the seven patients, and in all cases, in similar fashion to the urinary porphyrin values, either fell gradually during the first few days of therapy or rose slightly to a maximum of 134 i.u./l (normal range 5–35 i.u./l) in the case of aspartate transaminase, before eventually falling to normal or near normal levels. Serum iron levels were elevated in four patients before treatment, but did not respond in any apparently consistent way to therapy. A wide spectrum of pretreatment hepatic histological changes was noted. Two patients had virtually normal livers, one had mild inflammatory and fatty changes, one had fibrosis and three had precirrhosis or cirrhosis. There appeared to be no relationship between the severity of the liver changes and the degree of abnormality in liver enzyme or urinary porphyrin levels. There was, however, a suggestion of inverse correlation between the degree of the liver defect and the patients' stated alcohol intake. The two admitted heavy drinkers had fibrosis and the three who insisted that they had been non-drinkers had precirrhosis or cirrhosis. The remaining patient, a minimal drinker, had mild inflammatory and fatty changes. No adverse ophthalmological effects were noted.

DISCUSSION

Low-dose oral chloroquine therapy, which apparently acts in PCT by releasing tissue-bound hepatic porphyrin and facilitating its excretion in the urine (Scholnick, Epstein & Marver, 1973) has been effective and safe in our hands. Its efficacy has been a little less than previously reported by Malina and Chlumský (1973), Kordač and Semrádová (1974) and Kordač *et al.* (1977), but on the other hand better than found by Malkinson and Levitt (1980). Its safety has been proved in the large series of patients reported by the first three of the above authors. Other methods of therapy in PCT include strict alcohol abstention, which is also safe and may be tried particularly for milder cases (Brunsting, 1954; Ramsay *et al.*, 1974; Topi, Amantea & Griso, 1984), but is often ineffective or slow in effect, and repeated venesection, occasionally combined with low-dose oral chloroquine (Ippen, personal communication), which is also generally safe and is well established as an effective treatment (Ippen, 1961; Ramsay *et al.*, 1974). However, drawbacks of venesection are that it is sometimes difficult for the therapist, as in drug addicts with thrombosed veins, or unpleasant for the patient, and that it requires regular haematological monitoring. On the other hand, low-dose oral chloroquine therapy alone is easy and its relapse rate (Ramsay *et al.*, 1974) and duration of treatment (Epstein & Redeker, 1968; Ramsay *et al.*, 1974) are only marginally greater than with venesection. However, venesection should still be used if possible in patients with severe disease, marked signs of liver damage, unreliable personalities or unstable social backgrounds. This increases the likelihood that treatment will be carried out, facilitates accurate monitoring of treatment and progress and emphasizes the importance of the condition to the patient. However, in patients with mild disease, few signs of liver damage, reliable personalities and stable social backgrounds, low-dose oral chloroquine now appears to be the treatment of choice.

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Atypical psoriasis of the face and hands after PUVA treatment

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SUMMARY

In sixty patients with psoriasis, two types of lesions developed after repeated and prolonged courses of PUVA radiation. In sixteen patients, new lesions on the face resembled seborrhoeic dermatitis; in thirteen patients thick plaques on the dorsum of the hands developed insidiously over the years. There was no significant association between the two types of lesions and only the latter were dose-related. The appearance of recalcitrant psoriasis on the face and hands appears to be a complication of PUVA treatment.

The original reports of PUVA treatment for psoriasis described good results in almost all patients (Parrish *et al.*, 1974; Wolff *et al.*, 1976). In contrast, more recent publications recommend the addition of retinoids, methotrexate or local treatment to PUVA radiation, which implies the failure of PUVA treatment in a substantial number of patients. This tendency reflects the usual trend after publication of a new treatment, but possibly there is also a genuine decrease in the effect of PUVA after prolonged use. Lesions on the arms and legs in particular appear to become more resistant with repeated or prolonged treatment.

In this context we describe the persistance of old lesions or the appearance of new lesions on exposed areas after several courses of PUVA treatment.

METHODS

Sixty patients with episodes of severe psoriasis of all types were selected, who had a total dose of more than 250 J/cm^2 of PUVA radiation and who had been observed and treated in our clinic for at least 3 years (mean 9 years) before the advent of PUVA in 1977. Selection was otherwise random. Patients were treated according to the guidelines of the European co-operative trial (Wolff *et al.*, 1977; Henseler *et al.*, 1981) during exacerbations; we did not give maintenance treatment during remissions or minor relapses. The mean dose was 661 J/cm^2 (258–1852 J/cm^2). There were 36 men and 24 women, the mean age being 46 years (range 22–74 years). The

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mean duration of the disease was 23 years at the last PUVA-treatment. One clinician had treated all the patients since 1974.

In one-third of the patients the appearance or exacerbation of lesions on the exposed parts had been documented before our present prospective study was started, but from 1981 on, the type of lesions described here were systematically studied. Most of the patients also had other local or systemic treatment before or in between PUVA courses, but this was not given simultaneously with PUVA, except for lubricants and keratinolytic ointments.

RESULTS

Erythematosquamous facial lesions

Sixteen patients developed fine erythematosquamous lesions on the face, as described by Tegner (1983). In our patients, the upper cheek, especially the area adjacent to the nose, was almost invariably affected, followed to a lesser extent by the bridge of the nose, the glabella, the nasolabial folds and the periphery of the face. The eyelids, protected from PUVA, were not involved. Five patients (Table 1) occasionally had similar lesions before PUVA treatment. However, even in these cases they had become more severe and prolonged; a duration of several months was usual and five cases had facial lesions for 1 or 2 years. They were more resistant to local treatment than typical seborrhoeic dermatitis. Apart from the resemblance to 'seborrhoeic dermatitis' (Tegner, 1983) the appearance was occasionally suggestive of lupus erythematosus. The antinuclear factor was, however, negative in all patients as were the anti-dsDNA factor, anti-ENA factors and LE cells, in six cases. Biopsy was performed in five cases and showed the histological picture of seborrhoeic dermatitis or psoriasis without features of lupus erythematosus or polymorphous light eruption. There was no seasonal incidence.

Lesions on the back of the hands

Even more persistent lesions developed in thirteen patients on the knuckles, the dorsum of the first interphalangeal joints or the thenar eminence. Whereas the facial lesions were rather discrete, the lesions on the hands consisted of thick, infiltrated, deep red plaques or papules. There were no pustules and there was no relation with (pustular) palmar psoriasis. In eight patients lesions on the dorsum of the hands had been absent, or had been found only as transient episodes in short exacerbations before PUVA treatment (Table 1); in five others there was a definite, more persistent exacerbation. Patients who always tended to have prominent lesions on the hands are not included in the table.

TABLE 1. Lesions on the exposed parts in sixty psoriasis patients

	Erythemato-squamous lesions on the face	Persistent plaques on the dorsum of the hands
First appearance after PUVA radiation	11	8
Definite prolonged exacerbation	5	5
Total (males)	16 (10)	13 (10)

The lesions on the face and hands were dissimilar in course and clinical aspect and they concurred in three patients only. Thus, they were not significantly correlated ($P > 0{\cdot}7$, χ^2 test).

Further analysis of our data showed that the sex distribution and the age distribution (mean 42 years for patients with facial lesions, 43 years for those with hand lesions, total group mean 46 years) did not differ significantly from non-affected patients. The facial lesions were also not dose-related (mean 615 ± 394 J/cm^2, total group 661 ± 372 J/cm^2) in patients who had been irradiated with at least 250 J/cm^2, our criterion for selection. However, the mean dose in patients with hand lesions was 1013 ± 520 J/cm^2 and the correlation with high doses of PUVA proved significant ($P < 0{\cdot}05$) both in Student's *t*-test applied to the male group and in a multiple regression analysis of all the above-mentioned variables.

DISCUSSION

Psoriasis is usually relatively less prominent in areas exposed to sunlight, notably on the face. After repeated PUVA courses with a relatively high total dose, many patients tend to develop recalcitrant lesions in just these areas, notably in the 'butterfly' area which is otherwise typical for diseases caused by sunlight.

In the face, almost identical lesions were recently described by Tegner (1983). Hönigsmann (1983) commented on the fact that this side-effect had not yet been described and had also not been encountered in one of the largest and earliest groups of patients treated with PUVA in Vienna. It thus seems remarkable that this was by no means rare in Tegner's and in our study. We agree with Tegner (1983) that the facial lesions which she termed 'seborrhoeic dermatitis' and we consider as atypical psoriasis, do not appear during, but more usually after PUVA treatment and so may be missed. They may also not appear in centres where maintenance treatment is common practice. We support Tegner's suggestion that the entire face should be covered when PUVA is given in high doses.

We noticed a similar phenomenon, although with a different type of lesion, on the dorsum of the hands. Admittedly, in these lesions it is more difficult to distinguish between local persistence of lesions, exacerbations or new lesions. The lesions on the dorsum of the hands develop even more insiduously than on the face, get worse over the years and in our patients tended to be permanent. Even more misleading is the fact that both types of lesions tend to heal with repeated PUVA treatment; the local rebound occurs afterwards. It should also be noted that both types of lesions occurred in a group with severe, intractable psoriasis requiring rather high doses; we have rarely seen them in patients treated with less than 250 J/cm^2.

For obvious reasons, the connection with PUVA treatment can hardly be proved in a controlled study. Tegner (1983) has given some reasons why the association is probable. With regard to our study, it may be added that with such long mean duration of the disease and of follow-up in the same clinic, it is valid to use the long period preceding PUVA in the treated patients as a control (Glass, Wilson & Gottman, 1975). We have not noted any correlation with other treatments, including UV-B radiation. In patients who were treated subsequently with methotrexate or etretinate, we usually observed a beneficial effect on the PUVA-induced lesions.

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Pemphigus foliaceus induced by rifampicin

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SUMMARY

Pemphigus foliaceus was seen in a patient with pulmonary tuberculosis during rifampicin therapy. The skin lesions were eczematoid patches, flaccid bullae and crusted plaques on the trunk and extremities, histologically showing a high epidermal blister with acantholysis. The diagnosis was confirmed by immunofluorescent studies. After the patient discontinued rifampicin, the skin lesions cleared completely within 5 weeks without any systemic medication.

Rifampicin has been widely used for the treatment of tuberculosis since the 1960s. It does not often cause untoward effects, and when given in the usual dose, less than 4% of patients with tuberculosis develop significant adverse reactions (Mandell & Sande, 1980; Girling & Hitze, 1979). The most common are cutaneous and gastrointestinal and include facial flushing, rash, nausea and vomiting. Rifampicin-associated pemphigus has not yet been widely described and is presumably rare.

Since Degos *et al.* (1969) reported a case of pemphigus induced by D-penicillamine, many other cases of so-called drug-induced pemphigus have been reported, most of them being related to D-penicillamine (Santa Cruz *et al.*, 1981), but other drugs such as practolol (Amos, Brigden & McKerron, 1975), gold sodium thiomalate (Miyamoto & Maeda, 1978), captopril (Parfrey *et al.*, 1980), pyritinol (Santa Cruz *et al.*, 1981) and rifampicin (Gange *et al.*, 1976) have also been implicated.

To our knowledge, the present case of pemphigus induced by rifampicin is only the second reported in the English language.

CASE REPORT

A 49-year-old Korean man was seen in March, 1983, with an itchy eruption on his trunk and extremities. He first noticed the lesions on the upper abdomen 2 weeks previously and subsequently they gradually developed on his chest, abdomen, upper arms and flanks, and later on his thighs. Some of the lesions were blisters but others were erythematous patches. This

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patient had been found to be suffering from pulmonary tuberculosis in July, 1982 and for the following 8 months was on a regimen of 600 mg rifampicin, 400 mg isoniazid and 50 mg pyridoxine daily until just before his visit. He had not been taking any other drugs. A review of his medical history indicated that previous to suffering from pulmonary tuberculosis he had been in good health and had never had skin disease. His family history revealed nothing of note.

On physical examination the only pertinent findings were confined to the skin. On the chest, abdomen, flanks, upper arms and thighs there were scattered erythematous eczematoid patches, thumb-sized or smaller, mixed with flaccid bullae and crusted plaques with some erosions. In the lesional skin the Nikolsky sign was present. The patient did not have any oral lesions.

Laboratory studies including a complete blood count with differential, ESR and urinalysis were within normal limits. The pattern of serum protein electrophoresis, the values of liver function tests and thyroid function tests, blood urea nitrogen level, ASTO,VDRL, the serum concentrations of immunoglobulin, serum total haemolytic complement (CH50) and C3, C4 concentrations, antinuclear antibody and anti-DNA antibody, latex-fixing rheumatoid factor and cryoglobulin were all within normal limits or negative. Swabs taken from a flaccid bulla for bacterial culture were sterile. The result of sputum culture for *Mycobacterium tuberculosis* was negative at this time and the chest roentgenogram disclosed pulmonary tuberculosis of minimal activity.

Biopsy studies of the early lesional skin on the abdomen showed intra-epidermal bullae high in the epidermis, containing some neutrophils, eosinophils and acantholytic cells. A moderate perivascular infiltrate of lymphoid cells was seen in the upper dermis. Immunofluorescent studies were performed at the time of his first visit according to the established procedures (Jordon, 1980). The direct immunofluorescence showed epidermal intercellular deposits of IgG, especially in the upper part, and C3. However, uninvolved normal-appearing skin taken from the extensor surface of the forearm showed no specific deposit of immunoreactants. The indirect immunofluorescence of the serum, using normal human skin taken from the flanks of two healthy persons as the substrates, demonstrated circulating epidermal intercellular antibodies at a titre of 1 : 10. But when the patient's own skin taken from the same anatomical site was used as the substrate the titres were 1:40, in duplicate studies. Thus the biopsy and immunofluorescent studies were consistent with pemphigus foliaceus. Further studies of serum antibodies with different substrates could not be performed.

Suspecting a skin reaction caused by rifampicin, the patient was instructed to discontinue it, but to continue taking his other prescribed drugs and to apply antipruritic emollients topically. Two weeks later the skin lesions were much improved with only a few new lesions appearing, and the itching had decreased. Five weeks after stopping rifampicin the skin lesions had virtually cleared. Since the activity of his pulmonary tuberculosis was almost arrested, only isoniazid and pyridoxine were continued.

DISCUSSION

Drug-induced pemphigus or pemphigus-like syndrome has been well recognized in recent years. D-penicillamine-associated pemphigus was encountered most frequently, and in most patients the clinical and histological pictures were compatible with pemphigus foliaceus or pemphigus erythematosus. In most cases the skin lesions developed within 6–12 months of starting the D-penicillamine, as in the previous case induced by rifampicin (Gange *et al.*, 1976). Direct immunofluorescence studies showed depositions of IgG and complement in the intercellular areas of the epidermis in almost all cases in which it was performed. The great majority of the cases showed low titres of serum antibodies on indirect immunofluorescence, but the titre was negative in others. The forms of pemphigus induced by various drugs seem in general to be more benign than naturally occurring counterparts. In a few patients, however, the bullous disease has persisted for years and required vigorous treatment with systemic corticosteroids and other immunosuppressive agents.

It is not known how drugs such as D-penicillamine induce pemphigus, but it is possible that in the case of D-penicillamine the sulphydryl group of the drug changes the intercellular substance into an antigenic structure with subsequent antibody formation (Davies & Holt, 1976; Santa Cruz *et al.*, 1981; Ruocco *et al.*, 1982).

Even though the serum titre of intercellular antibodies was high (1:5120) in the first documented case of rifampicin-induced pemphigus, reported by Gange *et al.* (1976), the response to treatment with corticosteroid and the course of the disease suggest that it was more benign than is usual with pemphigus. These features are similar to those of other cases of drug-induced pemphigus and to those of the present patient. There seems no doubt that he had pemphigus foliaceus as shown by the clinical, histological and immunohistological features.

Epidermal cells have many free -SH residual proteins on the membrane or intercellular areas, especially in the granular layer or junctional zone of the living keratinocytes and the dead horny layer (Ogawa *et al.*, 1979). The existence of free -SH groups on the antigenic sites of the membrane of the epidermal cells seem to be necessary for their ability to bind and retain the pemphigus antibodies (Ogawa, Taneda & Morioka, 1978). According to this theory, it is possible that rifampicin may bind to the -SH groups of the epidermis *in vivo* and modify the intercellular antigen, thus being able to produce the antibody, especially in individuals who already have an underlying immunological susceptibility to it. The results of the indirect immunofluorescent studies showing that the patient's serum had more intense binding ability to his own skin may also support this hypothesis.

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British Journal of Dermatology (1984) **111**, 623–627.

Comment

DOES KISSING AID HUMAN BONDING BY SEMIOCHEMICAL ADDICTION?

Kissing is almost ubiquitous throughout the cultures of man (Eibl-Eibesfeldt, 1970), but what is it for? This paper proposes that kissing may be a mechanism by which semiochemicals are exchanged between human beings to induce bonding or love. From a review of the literature, a hypothesis is synthesized to suggest an explanation for the spectrum of bonding-related behaviour, from the warmth of affection to child abuse.

There may be as many definitions of *love* and *bonding* as writers on the subject (e.g. Murstein, 1971; Finck, 1891; Klaus & Kennell, 1982). This is yet another: one person's addiction to (or recognition of) a semiochemical(s) of another person.

Semiochemical is preferred to the more widely recognized term, *pheromone*, after the usage proposed by Mykytowycz because 'mammals display manifold responses to the same odour-signal depending on the behavioural situation, the physiological state of the individual and other factors controlled by the social and physical environments' (Mykytowycz, 1979).

Here olfaction as a possible semiochemical receptor mechanism will not be examined. The emphasis will be on the possibility of ingestion of a skin surface substance, sebum, during amorous contact. Skin sucking behaviour (kissing) allows only specific reception, without the inherent indiscrimination of olfaction. You can be smelled by a room full of people all at the same time, but kissing needs permission.

The American Psychological Association has abandoned the term *addiction*. It will be used in this paper following medical usage: physiological dependence on some agent (e.g. alcohol, drug). It should be pointed out that identifying *addiction* as a human response to semiochemicals is not crucial to the author's bonding hypothesis. *Recognition* could substitute.

SEBUM AS SEMIOCHEMICAL

Many substances, including sebum, are suspected to be semiochemicals in man (Comfort, 1974; Montagna & Parakkal, 1974). Yet, the role of human sebaceous glands remains obscure. 'The sebaceous glands are too numerous and active to be dismissed as trivial. Nor do they seem to be mere (vestigials) from earlier stages of evolution in which they were more important to the body' (Montagna, 1965).

Behavioural and physiological evidence suggests that sebum is a semiochemical in many species of mammals (Stoddart, 1976; Shorey, 1976), including primates (Epple, 1980). Marked differences are seen in sebum composition among animals (Strauss, Downing & Ebling, 1983). It is one of the most varied of all organic products in composition both inter-specifically and intra-specifically (Brown & Williams, 1972).

Man has more sebaceous glands than any other mammal (Montagna & Parakkal, 1974). The composition of human sebum varies from one anatomical location to another (Strauss, *et al.*, 1983; Greene *et al.*, 1970). Men are known to have more sebaceous glands than women (Strauss *et al.*, 1983; Montagna & Parakkal, 1974), paralleling the same dimorphism in mammals generally (Stoddart, 1974).

Sebum's composition and excretion rate change with age. The excretion rate is high shortly after birth, declining to the age of 1 year (Agache *et al.*, 1980). At puberty, its composition changes (Sansone-Bazzano *et al.*, 1979) and its excretion rate increases to a maximum in early adulthood. A significant drop in sebum production after the age of 49 in women is related to the menopause (Montagna & Parakkal, 1974).

Sebum excretion and sebaceous gland growth are stimulated by androgenic hormones (Strauss *et al.*, 1983; Montagna & Parakkal, 1974). Topical application of testosterone appears to have a direct effect, causing increased sebum production in prepubertal children (Montagna & Parakkal, 1974). Men appear to be producing sebum to maximum physiological capacity, for testosterone doses do not increase its production in normal mature males (Montagna & Parakkal, 1974). Sebum excretion drops precipitously with castration (Montagna & Parakkal, 1974).

SEBUM AND KISSING

While small sebaceous glands can be found associated with hair follicles over most of the surface of the body, very numerous, large sebaceous glands aggregate on the scalp, face, neck, female nipples and areolae, as well as contact surfaces of the sex organs (Montagna & Parakkal, 1974). With the important exception of the sex organs, these are skin surfaces where kisses are normally placed. In addition, sebaceous glands develop *de novo* inside the mouth beginning at puberty, in copious rosettes on the glabrous vermilion borders of the lips (especially the upper lip), and upon the surfaces of the buccal, oral, and gingival mucosae (Montagna & Parakkal, 1974). The licking and sucking of these same interior surfaces during a passionate ('French') kiss may effect removal and transfer of sebum.

The chemical characteristics of sebum tailor it for exchange during kissing behaviour. Sebum is extremely hydrophilic (Rothman, 1954, cited in Montagna & Parakkal, 1974). Puckering of the lips exposes the moist inside surface for contact. The extreme hydrophilicity of sebum could ensure its transfer from the skin surface where it was secreted to the lips kissing that surface. Kissing, especially erotic kissing of the mouth, face, neck, and chest involves moisture from saliva.

Kissing often incorporates a mild suction applied to the skin. This suction may help effect removal and uptake of the sebum covering that surface.

Indeed, the very presence of lips on the human mouth has puzzled one observer (Morris, 1967). If lips aid semiochemical uptake as suggested, a purpose for their natural selection seems evident.

During passionate heterosexual courtship behaviour (perhaps best described by the popular term *hot date*), increases in skin temperature have been recorded (Seeley *et al.*, 1980). As shall be shown, heat releases torrents of sebum.

SEBUM AND PASSION'S HEAT

Downing and his co-workers found a reservoir of sebum available for excretion in male facial skin (Downing, Stewart & Strauss, 1981). Heat is capable of emptying that reservoir, apparently, for Cunliffe and his associates found that 'the relationship between skin temperature and sebum excretion rate was highly significant, and heating and cooling produced changes on the order of 10% per 1°C' (Cunliffe, Burton & Shuster, 1970). This increase in sebaceous excretion with a rise in skin temperature is due to reduced sebum viscosity (Burton, 1970).

Sexual excitement increases circulation to skin surfaces and increases skin temperature, particularly during human sexual intercourse. These changes are described collectively as the *sex flush* (Masters & Johnson, 1966; Seeley *et al.*, 1980). Might an increased sebum excretion rate resulting from the heat of passion work to encourage bonding?

SEBUM AND BONDING IN PREGNANCY

Burton and his co-workers found that the sebum excretion rate increases during the last trimester of pregnancy, despite high levels of oestrogen, a hormone which normally inhibits sebum excretion (Burton, Shuster & Cartlidge, 1975). The elevated sebum excretion rate typical of pregnancy is also maintained during lactation. (Burton *et al.*, 1973).

These findings are consistent with the semiochemical-addiction bonding hypothesis and predict that pregnant and lactating women would be more 'kissable' than non-pregnant, non-lactating women. Enhanced bonding effort during the period when reproducing females are most vulnerable is logical.

SEBUM AND PARENT–INFANT BONDING

The vernix caseosa which covers the skin of neonates immediately post-partum has a sebaceous fraction from the large functioning sebaceous glands of the fetus (Montagna & Parakkal, 1974).

There is a 'period of 7 to 10 days after birth when the mother and baby are isolated together in 70% of human cultures' (Klaus & Kennell, 1982).

'In a study of two groups of primiparous mothers (Robson and Kumar, 1980) ($n = 112$ and $n = 41$) 40% recalled that their predominant emotional reaction when holding their babies for the first time was one of indifference. The same response was reported by 25% of 40 multiparous mothers. Forty percent of both groups felt immediate affection. *Most of the mothers in both groups had developed affection for their babies within the first week*' (Klaus & Kennell, 1982) (author's italics).

'Emanuel (1936) has shown that there is a marked post-natal sebum secretion. Our findings revealed that the levels obtained on the 4th day of life were comparable to those of young adults. This is an especially interesting fact which should be emphasized' (Agache *et al.*, 1980). Cross-cultural isolation, mothers falling in love with their babies, and a sebum excretion surge; could these occurrences be related?

SEBUM AND CHILD ABUSE

If mother–infant isolation facilitates bonding, following from her uptake of sebum from the infant through kissing, anything acting to separate mother from baby or otherwise acting to deter her from kissing her infant during this critical period, should be expected to have a negative effect upon bonding success.

'25 consecutive unequivocally abused children who had 1 or more siblings were selected from our referrals. Six factors emerged as highly significantly over-represented in the proband biography as compared with the control sibling group: abnormal pregnancy, abnormal labour or delivery, neonatal separation, other separation in the first six months, illnesses in the first year of life, and illness in the mother in the first year of life' (Lynch, 1975). Lynch believes child abuse results from bonding failure. Most, but not all, researchers agree (see Klaus & Kennell, 1982). Her findings are compatible with the semiochemical-addiction bonding hypothesis because her 'highly significantly over-represented' factors correlating with parental abusive behaviour would interfere with semiochemical uptake from the abused child by the parent. As sebum excretion declines to a base level at 1 year (Agache *et al.*, 1980), the probability of creating a successful bond between parent and child should also decline.

It is now the practice in adoption agencies to place babies with adopting parents as soon after birth as possible, 'the earlier the better' (Cady & Cady, 1956). Experience has also taught us to pity the plight of the older orphan all too likely to be doomed to grow up at arms length from his or her caretakers.

If love consists of a dependency upon a semiochemical commerce, understanding that process may give us the tools to reduce the risk of child abuse and other human behavioural maladies as well.

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British Journal of Dermatology (1984) **111,** 628.

Obituary

Roy Summerly M.B., Ch.B(Birm); FRCP, FRCPE

With the death of Roy Summerly on 2 April 1984, at the age of 51, British dermatology has lost one of its foremost clinicians; very many dermatologists will feel the loss of a friend.

Roy was consultant dermatologist based at the North Staffordshire Hospital Centre, Stoke-on-Trent, and senior lecturer in Dermatology in the Department of Postgraduate Medicine, University of Keele. He qualified in Birmingham in 1955 and after registration spent 2 years in the RAMC during which time he was clinical officer in dermatology and venereology. He opted for a career in dermatology and after initial medical and dermatology posts in North Staffordshire he was registrar and then senior registrar at St Thomas' Hospital, London; concurrently, he was also honorary clinical assistant at the Institute of Dermatology, St John's Hospital for Diseases of the Skin, London. In 1966, he was appointed consultant dermatologist based at the North Staffordshire Hospital Centre and rapidly became known to his colleagues as a physician of the highest calibre. He proved to have remarkable 'all round' qualities, being able to supervise a very busy clinical unit, undertake important basic clinical and laboratory research, and manage an enormous administrative load in his local health district, regionally and in dermatology at national level. In his early years as a consultant he carried out much of his laboratory research work in the MRC Unit of Experimental Pathology at the Medical School in Birmingham where he was successively research fellow and consultant; when the unit moved to the University of Keele he was appointed senior lecturer in dermatology. Such was the quality of his research that he was in great demand as a lecturer; he was elected Dowling Orator for 1973.

Roy's calm diplomacy, energy, attention to detail and approachability made him an ideal medical administrator. He was involved in a vast number of executive bodies in his local district and region, both in medicine generally, and dermatology. At various times in his consultant career he was an elected member of the governing committees of all the national dermatology organizations—British Association of Dermatologists, British Society for Investigative Dermatology, Royal Society of Medicine and Royal College of Physicians; shortly before his death he was made a member of the Specialty Advisory Committee of the Joint Committee on Higher Medical Training. He will be long remembered for this truly massive commitment to his profession, but the memory of his personal qualities outlives even these achievements. Certain medical schools are often praised for their ability to attract undergraduates to dermatology as a career. Less common knowledge is the fact that the clinical ability of Roy Summerly attracted at least twelve 'uncommitted' doctors undertaking general medical (pre-MRCP) training in North Staffordshire Medical Centre to take up dermatology—they are all now successful senior registrars or consultants; a wonderful testimony to his clinical acumen, teaching ability and infectious interest in his chosen field. His superb diagnostic ability and 'memory for cases' was common knowledge throughout the North and Midlands and to his peers far and wide—the 'Case for Diagnosis' at clinical meetings was often easy for him; he could always be relied on to quote similar cases or suggest previous literature or useful therapy.

Roy, his wife Myrtle, and in their younger days, children Jeremy and Kate, were very gregarious members of the 'dermatology family'. We share with Myrtle, Jeremy and Kate, profound grief and sorrow at Roy's death.

R.D.

British Journal of Dermatology (1984) **111**, 629–631.

Correspondence

'Coudability': a new physical sign of alopecia areata

Sir, The diagnosis of classical alopecia areata is simple but problems may arise with diffuse disease, in which the whole scalp may be involved but without discrete patches, and alopecia areata in which the margin of the patch is vague because short and long hairs are mixed. In addition to the characteristic hair stumps and patent follicular orifices there is another feature which may help diagnosis, especially where the margins of the patches are vague. I have used it for many years and it is probably worth reporting as others seem not to know of it.

In classical alopecia areata the disorder affects most of the follicles in the patch and the hairs snap off, sometimes (but especially in children) leaving dark dots at the follicular orifices and, more often, the better known and loved exclamation-mark-shaped stumps. In such affected follicles there appears to have been a short period of growth of poor hair which then snaps, the length of the stump being dictated by duration of the period between onset of disease and cessation of follicular growth. (Interestingly, the appearance of the exclamatory stump is a composite of three easily discernible factors: (1) loss of pigment proximally which makes the proximal shaft look narrower than it actually is; (2) splaying of the zone of rupture which looks even wider because of refraction of light; (3) a progressive narrowing of the shaft which is therefore less than suggested by appearances.) In the more subacute condition and in some follicles which are only affected partially or transiently, the hair can be seen to continue to grow. This results in a short length of poor shaft, partly depigmented and narrowed often with localized splaying where the hair has begun to split. Sometimes a single hair can be seen to be affected several times along its length indicating episodic disease and recovery. More often there is a single area of weakness which corresponds to a single episode and which is the basis of the physical sign I am at last ready to describe.

The sign is of a normal-looking hair which easily can be made to kink when bent or pushed inward, the kink corresponding to the shaft defect produced by the episode of alopecia areata. Often such kinks occur 5–10 mm above the scalp, presumably because the follicle is no longer active and the stationary shaft defect has not yet revealed itself by snapping. Less commonly it is because the follicle has recovered but the hair has not yet grown out sufficiently to localize a fracture. After snapping, some of these hairs continue growing and this explains the long exclamation hairs—often up to several centimetres in length—seen occasionally in alopecia areata, but more often found if looked for. When irregularity of shape or vagueness of outline of the lesion causes diagnostic doubt, inward movement of the hair will invariably produce a sharp kink, usually several millimetres from the scalp, which reveals the site of affection of hairs caught by alopecia areata. The follicles of such hairs can be removed with a forceps applied below the kink and usually show the characteristic anagen atrophy. I have not seen such hairs in other diseases. The appearance of the kink gives the hair the shape of a coudé catheter (Bailey, Bishop & Morson, 1958). For this reason, and because the adjective 'kinky' has already been applied to the hair in copper deficiency, 'coudability' would be an appropriate term for this sign of the hairs of alopecia areata.

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Effectiveness of dapsone in Behçet's disease

Sir, We were pleased that Dr Sharquie found dapsone to be effective in treating Behcet's disease (Sharquie, 1984). We previously found similar improvement in one patient (Convit, Goihman-Yahr &

Rondón-Lugo, 1972). On discontinuing the drug, lesions reappeared, but improved again when dapsone was readministered. Our case also had a thymoma and hypogammaglobulinaemia. We have also found dapsone to be effective in cases of severe recurrent oral aphthae (unpublished data).

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Ritodrine therapy associated with remission of pemphigoid gestationis

Sir, We would like to report a case of complete remission of severe pemphigoid gestationis (PG, also called herpes gestationis) during treatment with ritodrine.

Case history. A 32-year-old woman developed PG during the 34th week of her first pregnancy in 1980. The intensely itchy and blistering eruption rapidly became generalized, and needed prednisolone 20 mg daily for effective suppression. The PG continued actively throughout the latter stages of pregnancy and finally abated 2 months post-partum. A normal male infant, birth weight 2·94 kg, was born at term. Her second pregnancy terminated in an intra-uterine death at 28 weeks without evidence of PG. Her third and latest pregnancy progressed normally until the 24th week when PG recurred as in the first pregnancy. Histology again confirmed the diagnosis and direct immunofluorescence showed a thick band of C3 deposition along the basement membrane of para-lesional skin. Circulating PG factor was not detected. On this occasion she required prednisolone in an initial daily dose of 40 mg and subsequently 25 mg to maintain even reasonable control. During the 32nd week she was admitted with abdominal cramps and vaginal bleeding, and was commenced on intravenous ritodrine (Yutopar®) for premature labour. Within a few days all itch and evidence of PG had disappeared and within 2 weeks the prednisolone had been completely tailed off. For the remainder of her pregnancy she was maintained at home on oral ritodrine with normal progression of the pregnancy and normal oestrogen levels. She was readmitted at the 37th week in labour and gave birth to a normal female infant, birth weight 2·66 kg. A recrudescence of PG occurred post-partum when she was on no further therapy. This responded to a short course of prednisolone and settled within a month.

Ritodrine is a beta-mimetic drug and predominantly a β_2-receptor agonist. It is prescribed for patients in premature labour to reduce uterine contractility and to enable fetal lung maturation.

The complete remission of PG during ritodrine treatment in our patient is particularly striking by comparison with the very active PG which occurred during the same stage of her first pregnancy. PG may remit spontaneously in late pregnancy, perhaps due to hormonal changes (Holmes *et al.*, 1982), but this is unlikely to have been the case in our patient because of the unabated activity of the PG during the final phase of her first pregnancy.

PG is rare, occurring in about 1 : 10,000 deliveries. We would be interested to know if a therapeutic effect of ritodrine has been observed by others, and we suggest a short trial of this drug, which is safe in pregnancy, in cases of PG requiring systemic steroids.

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Treatment of lymphomatoid papulosis with methotrexate

SIR, Drs Wantzin and Thomsen report the treatment of three patients with lymphomatoid papulosis by methotrexate (*Br. J. Dermatol.* 1984, **111**, 93). The majority of patients exhibiting this histological picture were originally (Verallo & Haserick, 1966) and continue to be considered a variant of pityriasis lichenoides (Black & Wilson Jones, 1972; Demis *et al.*, 1974; Everett & Headington, 1978; Fitzpatrick *et al.*, 1979; Lambert & Everett, 1981). Consequently, methotrexate has been employed, at least in the United States, in the treatment of lymphomatoid papulosis since it was first reported effective in pityriasis lichenoides by Cornelison *et al.* (1972). We have treated eight patients with lymphomatoid papulosis who have been followed from 4 to 9 years. Four did not require further therapy after 6 to 12 months of low-dose methotrexate therapy. An additional four have required intermittent treatment to prevent recurrences. One patient, later diagnosed as 'cutaneous lymphoma' elsewhere, continues to respond completely to small doses of methotrexate. Our patients have required schedules of 2·5–5 mg at 2- to 6-week intervals up to 15 mg at 7–10 day intervals.

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Book Reviews

Mould Allergy. Edited by Y.AL-DOORY AND J.F.DOMSON (1984) Philadelphia: Lea and Febiger. Pp. 287. Price £31.25.

In their preface the editors of this multi-author book point out the paucity of information on the role of fungi in allergic disorders in most textbooks dealing with this subject. The aim of the work is to bring together available data in a convenient and accessible form.

The book starts promisingly with a number of interesting chapters, of which those on sampling techniques and conidia are of particular interest. However, subsequent sections are a little disappointing in that some of the subjects are covered in insufficient detail for the clinician. The clinical sections on allergic bronchopulmonary aspergillosis is an example. The work ends with a useful review of the effects of climate on fungal allergy and spore dispersal.

While this book may have a number of sections of value to chest physicians and clinical immunologists it is unlikely to be of great appeal to most dermatologists unless they have a specific interest in this field. The subjects most relevant to skin disease discussed include intradermal trichophytins, immunological tests, vaccination and a brief review of immunity to Candida. However, most of these are considered in greater depth elsewhere.

Generally the book is well presented and contains 287 pages including black-and-white photographs and illustrations.

R.J.HAY

Dermatology. An Illustrated Guide. By L.FRY (1984) 3rd edition. London: Butterworth/Update. Pp. 192, 553 illustrations. Price £19.50.

This book is obviously proving very popular; three editions in 10 years is an achievement of which any author should be proud. As one might expect with such a successful product the format is unaltered. A number of new topics have been added such as hyperhidrosis and Kaposi's sarcoma but overall this edition is only about 20 pages longer than its predecessor. Essentially it is an atlas of colour photographs with an accompanying text encompassing such subjects as morphology and treatment. The text reflects sound clinical practice and cannot be seriously faulted. The quality of the photographs is good overall but some are badly over-exposed. Why else when playing my favourite game of 'guess the diagnosis from the pretty picture' would a man of my experience misdiagnose eczema herpeticum as lichen planus? It is a game I would like to recommend to the author. Not only will it prompt him to get better pictures of such conditions as herpes gestationis (and eczema herpeticum) but it will also help him to spot the scabetic burrow in necrobiosis lipoidica (p. 129) and compare it with the yellowish thinning of the skin seen in a typical case of scabies (p. 137). One is entitled to be churlish when these four spoiled my uncritically self-awarded 100% pass rate.

Overall though, the book deserves a highly commended rosette in any dermatological Crufts as being one of the best of an increasingly popular and hardy breed.

F.A.IVE

Carbon Dioxide Laser Surgery. By I.KAPLAN AND S.GILER. (1984) Berlin: Springer. Pp. 206. Price DM 198.

This book consists mostly of colour photographs showing what is possible with the carbon dioxide laser in all aspects of surgical practice. Although there is a sizable section devoted to cutaneous lesions, there are some surprising omissions. For instance, only passing mention is made to the use of carbon dioxide laser in the treatment of portwine stains.

I have several reservations about this book. It is written as though there is only one manufacturer of carbon dioxide lasers. There is very little text, so that anyone wanting to get a clear theoretical background on lasers would have to look elsewhere. However, there is a useful list of references at the end of the book.

My review copy was badly printed and the preface and part of the table of contents were both unreadable. The book may be useful for a quick superficial glimpse into this treatment area, but is not likely to be of continual help as a source of reference to those intending working in the field.

J.A.Cotterill

Where No Skin Specialist is Available. P.N.Behl, assisted by A.Aggarwal. New Delhi, India: The Skin Institute and School of Dermatology.

As pointed out by the authors, there is clearly a lack of general practitioners and certainly dermatologists in any Indian district outside the main town areas. It is therefore difficult for a large number of patients with skin conditions to get expert medical advice. This booklet is primarily meant for community workers and basic rural doctors who will often have to deal with these problems. It contains a good deal of dermatological common sense and will be of considerable value in the country districts of India and in many other countries. Containing equivalent Indian names for disorders and medicaments, it nostalgically recalls the attraction of India to those who have travelled there.

Some customs described may surprise our readers and one wonders about their scientific justification. For example, it is difficult to understand what is prevented by regularly oiling the foreskin and under surface of the prepuce! If a patient is laboured with instructions which are impossible to carry out, it is no longer the fault of the advising physician that a cure is not attained. Under the 'General Case' section it is recommended that the diet should include 'meat, fish, milk, milk products, green vegetables and fruits'!

I think Drs Behl and Aggarwal are to be congratulated on producing this booklet which will do a great deal of good and which is distributed free of charge.

Robert P.Warin

British Journal of Dermatology (1984) 111, 634.

News and Notices

Annual Meeting of the B.A.D., London, July 1985

The 65th Annual Meeting of the B.A.D. will be held in London from July 3 to July 5 1985, at Kensington Town Hall and St George's Hospital, under the Presidency of Dr K.V.Sanderson. Those wishing to present a paper or a scientific exhibit at the Meeting should submit an Abstract (six copies) of not more than 200 words to the Honorary Secretary, Dr G.M.Levene, at 7 John Street, London, WC1N 2ES before 4 February 1985.

Immunodermatology Symposium, Milan, September 1986

The Third Immunodermatology Symposium will be held in Milan, Italy on 21–23 September 1986. For further information contact: Professor R.Caputo, Clinica Dermatologica Prima, Universita di Milano, Via Pace, 9, 20122 Milano, Italia.

New Trends in Allergy, Munich, July 1985

The Second International Symposium on 'New Trends in Allergy' will be held in Munich on 26–28 July 1985. For further details please contact: Professor J.Ring, Dermatologische Klinik und Poliklinik der Ludwig-Maximilians-Universitat, Frauenlobstrasse 9-11, D-8000 Munchen 2, West Germany.

A two-day course in Paediatric Dermatology, Liverpool, May 1985

The third 2-day course in Paediatric Dermatology arranged by the University of Liverpool will be held in Liverpool on Thursday, 16 May and Friday 17 May 1985. The course will be aimed at Registrars, Senior Registrars and newly appointed Consultants and there will be a limit of 12 participants. Further details from Dr J.L.Verbov, c/o Postgraduate Dean's Office, Faculty of Medicine, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.

Skin Club Meetings 1984/1985

29 November 1984, London. 'Biological and Clinical Aspects of Hair Growth Control.' Organizer: Dr R.F. Oliver.
28 February 1985, Wellcome Research Laboratories, Beckenham, Kent. 'Leukotrienes.' Organizer: Dr H.J.Yardley.
30 May 1985, Smith and Nephew Research Ltd., Gilston Park, Harlow, Essex. 'Wound Healing.' Organizer: Dr D.F.Webster.
11 July 1985, London. 'Scleroderma.' Organizer: Dr P.J.Dykes.

For further details please contact Dr P.J.Dykes, Department of Medicine, Section of Dermatology, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN. Telephone: Cardiff (0222) 755944, Ext. 3146.)

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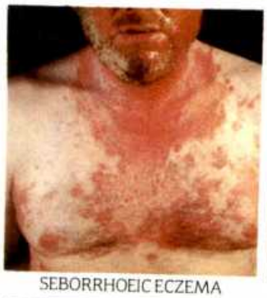
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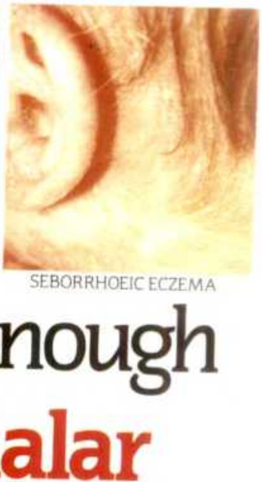
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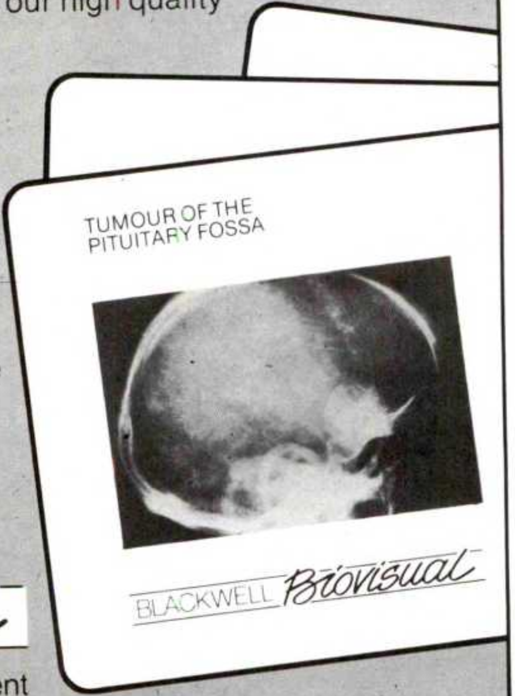

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Manuscripts should be sent to the Editor, Dr J.L.Burton, Dermatology Department, Bristol Royal Infirmary, Bristol BS2 8HW. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. A duplicate copy, complete with figures, tables and references, should be sent, since in many cases two referees are consulted. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

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kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
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microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
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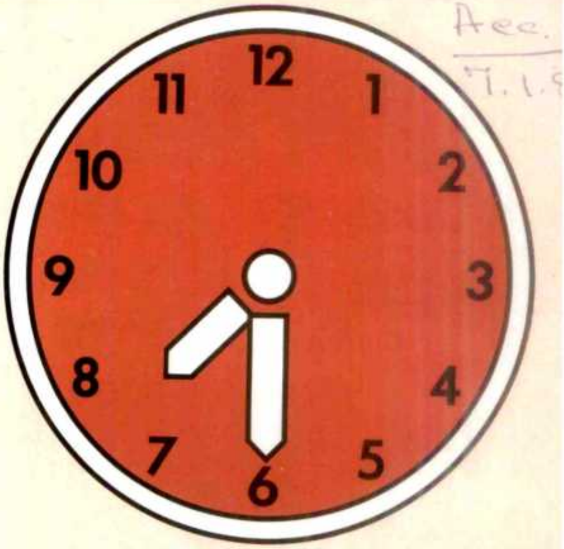

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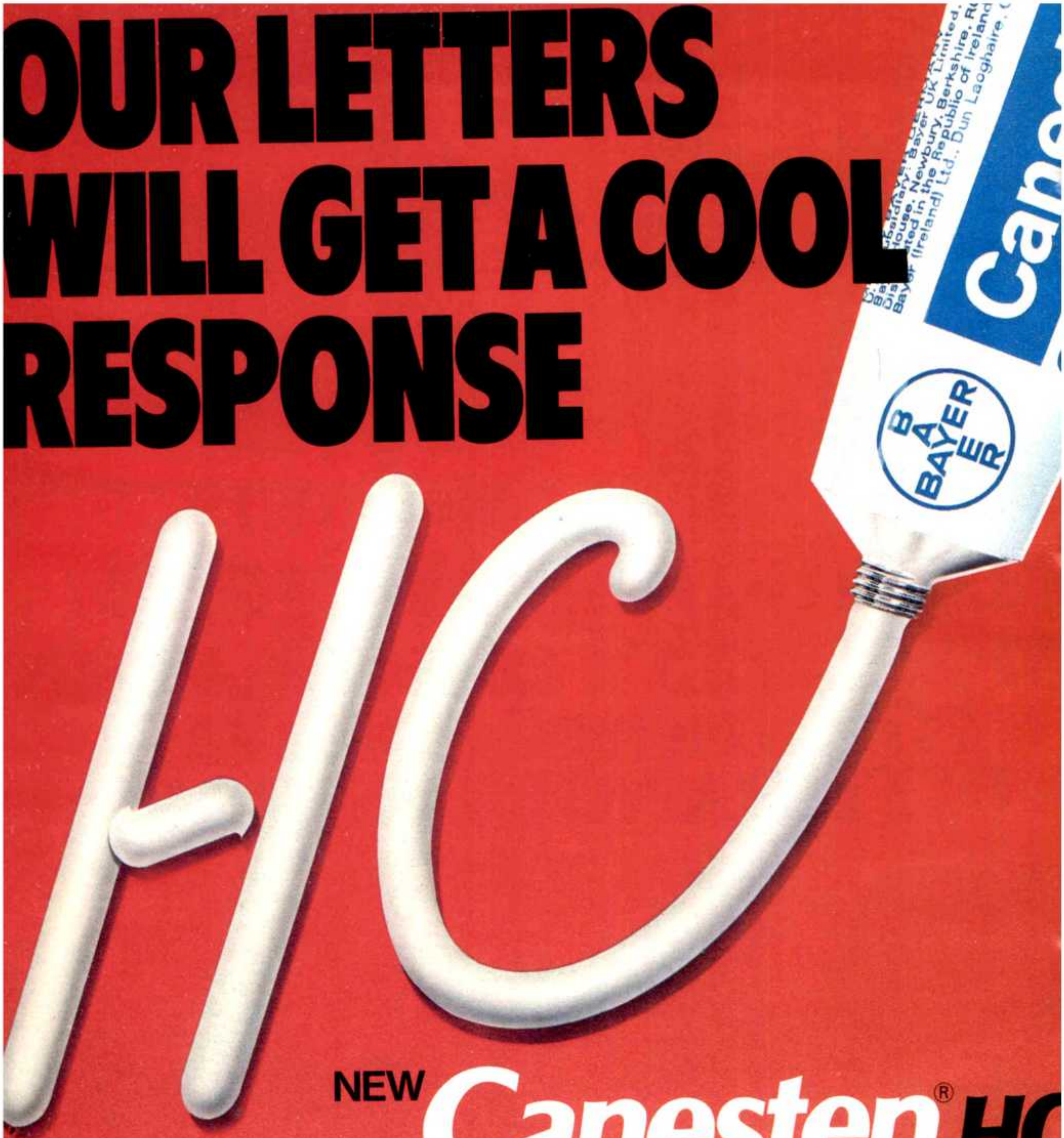

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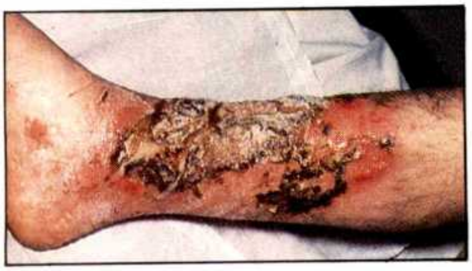
References 1. Cutis (1978) **21,** 403. 2. Curr. Med. Res. Opin (1977). **4,** 650. 3. Curr. Ther. Res. (1978) **24,** 37. 4. Drugs (1978) **16,** 302.

Ulcer healing is



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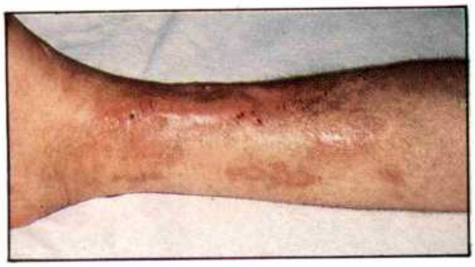

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rence Number: 29/0165. **References:** 1. Ormiston M.C., *et al*, in Fischer H. eds
dexomer Iodine. Stuttgart: F. K. Schattauer. 1983:63. 2. Moberg, S., *et al*,
Amer.Geriat.Soc.1983; 31:462. 3. Skog, E., *et al*, Br. J. Dermatol 1983; 109:77.

Further information available on application
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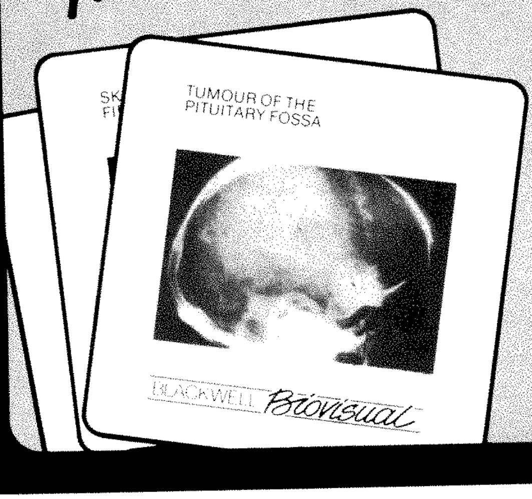

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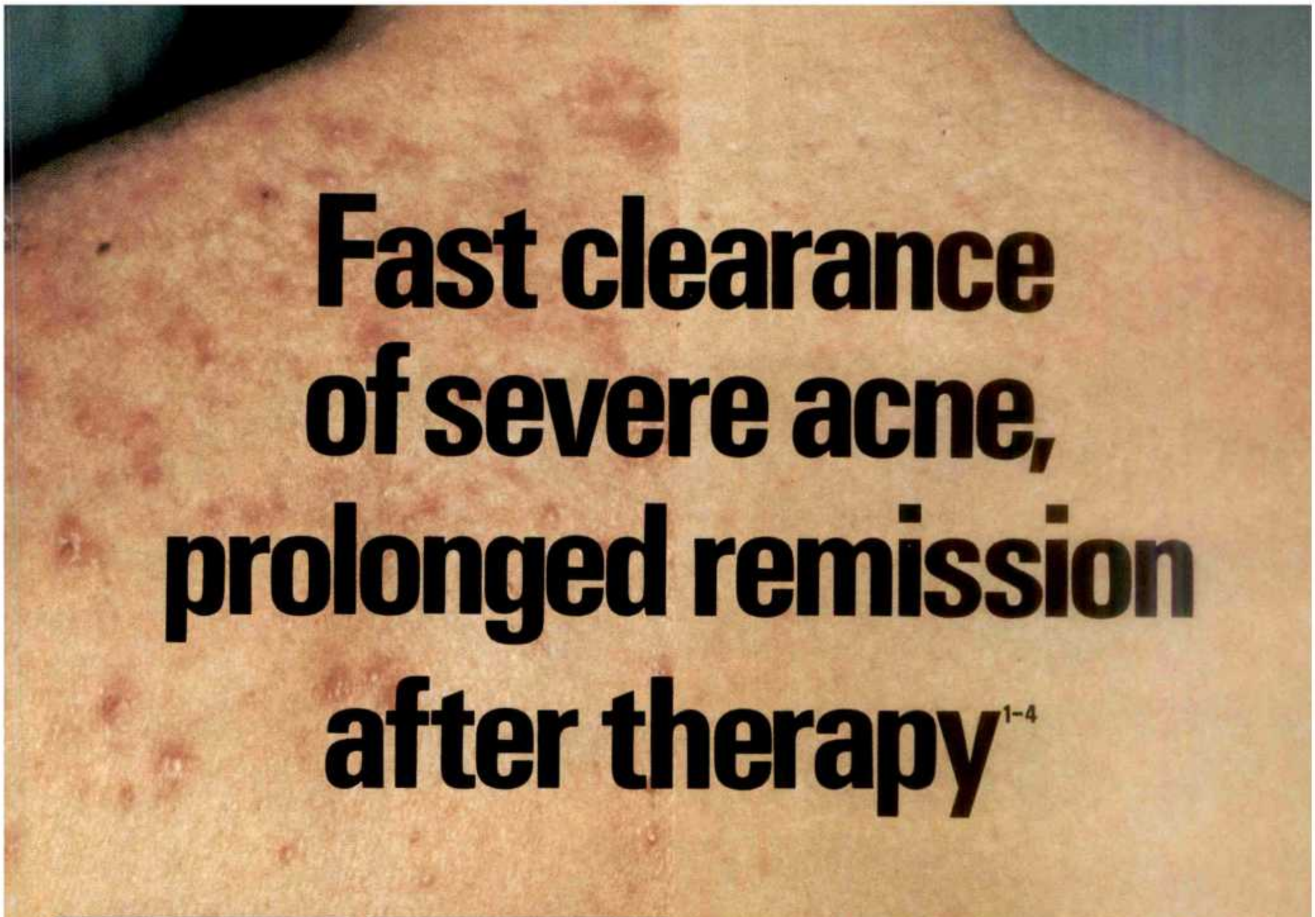

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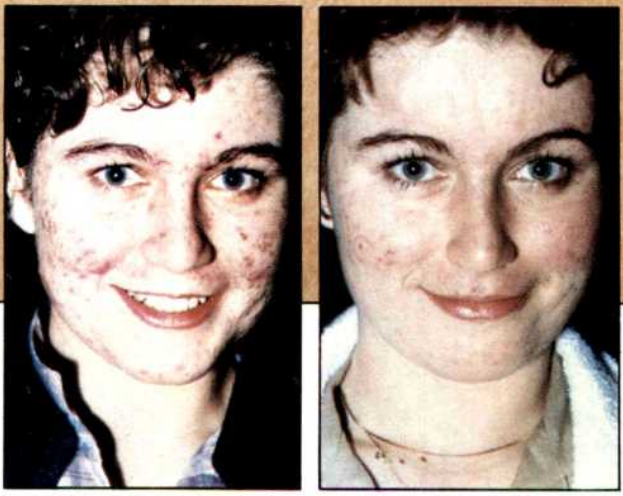

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1. In: "Retinoids: Advances in Basic Research and Therapy". Eds. Orfanos, C.E. *et al.* Springer-Verlag, Berlin, 1981, p.219.
2. Brit.J.Derm., 1983, *108*, 333.
3. J.Amer.Acad.Derm., 1982, 6, 735.
4. J.Amer.Acad.Derm., 1982, 6, 766.
5. J.Amer.Acad.Derm., 1983, 9, 629.

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British Journal of Dermatology (1984) 111, 635–645.

Clinical and Laboratory Investigations

Characterization of benign cutaneous lymphocytic infiltrates by monoclonal antibodies

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SUMMARY

Skin biopsies from nine patients with a histological and/or clinical diagnosis of cutaneous lymphocytoma, lymphoplasia or Jessner's lymphocytic infiltrate were examined by immunoenzymatic labelling with a panel of monoclonal antibodies against lymphocytes and accessory cells.

Similar cellular constituents were demonstrated in the biopsies from three patients with lymphocytoma, two with lymphoplasia and two with atypical lymphocytes, but a protracted benign clinical course. The infiltrates from these patients consisted of T cells, Langerhans cells, related HLA-DR positive dendritic dermal cells and clusters of polyclonal B cells. In four patients, the B cell clusters contained B cell accessory follicular dendritic cells, and thereby closely resembled the B cell follicles seen in lymphoid organs. The T cells were predominantly T helper/inducer cells and in all patients the T cells expressed HLA-DR.

One patient diagnosed as lymphocytoma cutis differed from the other patients by having no detectable B cells. One patient with Jessner's lymphocytic infiltrate differed from the other patients by having a marked relative predominance of T suppressor/cytotoxic cells.

These data suggest that cutaneous lymphocytoma and lymphoplasia are basically similar disorders which may be considered to be exaggerated immune responses, whereas Jessner's lymphocytic infiltrate may be a separate entity. Immunological analysis may assist in establishing a definite diagnosis in cases of lymphocytoma or lymphoplasia with atypical cytological features.

Lymphocytoma cutis, cutaneous lymphoplasia and Jessner's lymphocytic infiltrate form part of the benign lymphocytic skin infiltrates (Clark *et al.*, 1974; From, 1979; Mach & Wilgram, 1966; Lange Wantzin *et al.*, 1982). These disorders form plaques or tumours which arise

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Correspondence: Elisabeth Ralfkiær, Department of Pathology, The Finsen Institute, 49 Strandboulevarden, DK-2100 Copenhagen ø, Denmark.

spontaneously or supervene during the course of pre-existing inflammatory skin disease (Mach & Wilgram, 1966). The lesions may persist or recur for long periods and may closely resemble malignant lymphomas (From, 1979). Certain histological features favouring a benign diagnosis (e.g., a mixed infiltrate, germinal centre formation, fibrosis, vascular proliferation) have been identified in some series of patients (Evans, Winkelman & Banks, 1979; MacDonald, 1982). However, these features are inconstantly present and in the individual patient a definite diagnosis frequently cannot be made without several years of follow-up.

Some insight into the nature of the infiltrating cells in these disorders has been obtained by immunological analysis of cell suspensions with rosetting and immunostaining techniques, and the suggestion has been made that lymphocytomas represent exaggerated immune responses (Knowles, Halper & Jakobiec, 1982).

In this study, the infiltrating cells in cutaneous lymphocytoma, lymphoplasia and Jessner's lymphocytic infiltrate have been characterized with an extensive panel of recently developed monoclonal antibodies, using the technique of *in situ* immunoenzymatic labelling of frozen sections (Stein *et al.*, 1982) which preserves the tissue architecture and prevents enrichment of particular cell subsets.

METHODS

Patients fulfilling either one or both of the following two sets of criteria were selected for study: (1) a clinical diagnosis of benign lymphocytic infiltrate in the presence of typical histology; (2) a clinical diagnosis of benign lymphocytic skin infiltrate, and a subsequent benign clinical course for at least 5 years. The latter criteria were included because it may be extremely difficult, and sometimes impossible, to establish a definite diagnosis of benign lymphocytic skin infiltrate on histological criteria (Clark *et al.*, 1974; Evans *et al.*, 1979; Lange Wantzin *et al.*, 1982; MacDonald, 1982). Nine patients who fulfilled these criteria were studied. Their clinical data are summarized in Table 1. No attempt was made to discriminate between lymphocytoma cutis and cutaneous lymphoplasia on clinical criteria (From, 1979). The past history was unremarkable in six patients. In one patient (No. 1) lesions developed 8 years subsequent to the diagnosis

TABLE 1. Clinical data from nine patients with benign lymphocytic skin infiltrates

Patient	Age	Sex	Clinical diagnosis*	Skin lesion	Solitary	Multiple	Therapy†	Present status‡	Follow-up (month)
1	13	F	BL	Tumour		+	XRT, G(s)	A^+	12
2	30	F	BL	Tumour	+		XRT, G(1)	A^0	15
3	35	M	BL	Tumour		+	XRT	A^+	72
4	46	M	BL	Tumour		+	XRT	A^+	132
5	53	M	BL	Tumour		+	XRT, MTX	A^0	165
6	57	M	JLI	Plaque		+	C	A^+	57
7	67	M	BL	Tumour	+		XRT	A^+	12
8	73	M	BL	Tumour		+	XRT, (G(1)	A^0	132
9	79	M	BL	Tumour	+		P	A^0	8

* BL: benign lymphocytoma or lymphoplasia; JLI: Jessner's lymphocytic infiltrate.

† XRT: X-ray therapy; G(s): systemic glucocorticoids; G(1): intralesional glucocorticoids; MTX: methotrexate; C: chloroquine orally; P: penicillin orally.

‡ A^+: Alive with active disease; A^0: alive in remission.

of atopic dermatitis, in another (No. 8) 2 years subsequent to the diagnosis of nodular prurigo and in the remaining patient (No. 9) 1 month after local trauma.

The patients have been followed for periods of between 8 and 165 months (median: 57 months) without evidence of malignant lymphoma. Chest X-rays and blood tests (Hb, total leukocyte counts, differential counts, and serum immunoglobulins) were normal in all patients, except in the patient suffering from atopic dermatitis who showed eosinophilia and a markedly elevated level of serum IgE. Lymphangiography and lymph node biopsy were performed in two patients (Nos 1 and 5) because of regional lymph node enlargement. Lymphangiography was normal in both patients. Lymph node biopsies demonstrated benign hyperplasia in the form of dermatopathic lymphadenitis.

Seven patients have been treated with one or more courses of radiation therapy supplemented with systemic or intralesional glucocorticoids (Nos 1, 2 and 8) or methotrexate (No. 5). The remaining two patients have received chloroquine (No. 6) or penicillin (No. 9) orally. In all patients lesions disappeared initially, but in five patients (Nos 1, 3, 4, 6 and 7) they recurred 3–4 months after completion of therapy and have persisted 12–132 months (median: 57 months) after diagnosis.

Histological evaluation

Serial sections of a total of forty-five formalin-fixed, paraffin-embedded, haematoxylin–eosin stained skin biopsies were examined. The infiltrates were classified according to criteria described previously by Clark *et al.* (1974) and subsequently modified by From (1979). Distinction was made between dense follicular infiltrates (lymphoplasia), dense non-follicular infiltrates (lymphocytoma) and other variants including lymphocytic infiltrates of the reticular dermis of the Jessner–Kanof type. Cases with cytological atypia of the infiltrating lymphocytes were grouped separately (uncertain histological diagnosis).

Immunological analysis

Fresh tissue specimens. Skin punch biopsies (4 mm) were obtained before institution of therapy (Nos 1, 2, 5 and 9) or during disease relapse 3–4 months after completion of therapy (Nos 3, 4, 6, 7 and 8) (see Table 1). Different areas of involvement were examined in three patients (Nos 1, 4 and 5) and altogether fourteen specimens were available for immunological analysis.

Monoclonal antibodies. The reactivities of the antibodies used in this study are summarized in Table 2.

Preparation and immunoenzymatic staining of frozen sections. Biopsies were frozen and stored in liquid N_2. Cryostat sections (6 μm) were lyophilized at -20°C for 2–4 h, fixed in acetone for 10 min, and incubated with monoclonal antibodies for 30 min (Stein *et al.*, 1982). The sections were then washed in Tris-buffered saline and labelled with peroxidase-conjugated rabbit anti-mouse immunoglobulin (Dakopatts) diluted 1 : 10 with phosphate-buffered saline containing 33% (v/v) normal human serum (30 min). The peroxidase reaction was developed using 3-ethyl-9-amino-carbazole and H_2O_2 as substrate. The sections were counterstained with Harris' haematoxylin and mounted in Aquamount®.

The number of Leu 3-, Leu 2-, anti-kappa- and anti-lambda-positive cells was estimated by counting labelled cells relative to the total number of nuclei within the fields of a 5 × 5 mm ocular net at ×400 magnification. The number of epidermal Langerhans cells were counted as described previously (Harrist *et al.*, 1983).

TABLE 2. Monoclonal antibodies

Antibody	Specificity	References	Commercial source*
Leu 1/Lyt 2	Pan T cells	Martin *et al.* (1981)	BD/NEN
Leu 5/Lyt 3	Pan T cells (E-rosette receptor)	Kamoun *et al.* (1981)	BD/NEN
Leu 4	Peripheral T cells	Reinherz & Schlossmann (1980)	BD
Leu 3	T-helper/inducer cells	Ledbetter *et al.* (1981)	BD
Leu 2	T-suppressor/cytotoxic cells	Ledbetter *et al.* (1981)	BD
OKT6	Cortical thymocytes, Langerhans cells	Reinherz & Schlossmann (1980) Fithian *et al.* (1981)	Ortho
OKT9	Transferrin receptor	Terhorst *et al.* (1981)	Ortho
OKT10	Precursor cells, activated B and T cells	Terhorst *et al.* (1981)	Ortho
Leu 7	Natural killer cells	Abo & Balch (1981)	BD
B1	Pan B cells	Stashenko *et al.* (1980)	CC
To15	Pan B cells	Stein *et al.* (1982)	DAKO
R4/23	Dendritic reticulum cells	Naiem *et al.* (1983)	DAKO
To5	C3b-receptor	Gerdes *et al.* (1982)	DAKO
MO2	Monocytes	Todd, Nadler & Schlossman (1981)	CC
CR3/43	HLA-DR	Gatter *et al* (1982)	DAKO
M 702	IgM	Mason & Stein (unpubl.)	DAKO
M 703	IgD	Mason & Stein (unpubl.)	DAKO
N 10/2/27	Lambda	Mason & Stein (unpubl.)	DAKO
Anti-kappa	Kappa	—	BD

* Becton-Dickinson (BD); New England Nuclear (NEN); Ortho Diagnostics (Ortho); Coulter Clone (CC); DAKOPATTS (DAKO).

Control preparations. The number of OKT6 reactive Langerhans cells in the epidermis of six specimens from normal skin was estimated as indicated above. Sections of human hyperplastic lymph nodes or tonsils were stained as positive controls. Negative controls were performed by omitting the primary monoclonal antibodies.

RESULTS

Histological evaluation of skin biopsies

The data from routine histology of skin biopsies are summarized in Table 3. Four patients (Nos 1, 4, 7 and 9) demonstrated dense non-follicular infiltrates consisting of lymphocytes and histiocytic cells as well as occasional neutrophilic and eosinophilic granulocytes and plasma cells. The infiltrates demonstrated a distinctive grenz zone, were generally well-demarcated from the surrounding collageneous tissue, and spared blood vessels and appendages. The cells were cytologically benign with no pleomorphism and only occasional normal mitotic figures. In these patients a histological diagnosis of lymphocytoma cutis was therefore made. Two patients (Nos 2 and 8) demonstrated similar changes, but additionally their infiltrates contained germinal centres (Fig. 1). Consequently, these two patients were diagnosed as dense follicular infiltrates consistent with lymphoplasia. Two patients (Nos 3 and 5) demonstrated the same histological changes as those patients diagnosed as lymphocytoma cutis but some sections demonstrated areas with cytologically atypical cells (Fig. 2). Although both patients have had a

TABLE 3. Histopathological findings in skin biopsies from nine patients with benign lymphocytic skin infiltrates

	Patients								
	1	2	3	4	5	6	7	8	9
Infiltration									
Epidermis	—*	—	—	—	—	F	—	—	F
Appendages	—	—	—	—	—	F	—	—	—
Grenz zone	+	+	+	+	+	F	+	+	—
Upper dermis	D	D	D	D	D	F	D	D	D
Lower dermis	D	D	D	F	F	F	D	F	D
Germinal centres	—	+	—	—	—	—	—	+	—
Granulomas	+	—	—	—	—	—	—	—	—
Cell types									
Lymphocytes	+	+	+	+	+	+	+	+	+
Histiocytes	+	+	+	+	+	—	+	+	+
Others†	G,P	E	E	P	E,P	—	—	E,P	E,P
Cellular atypia	—	—	+	—	+	—	—	—	—
Fibrosis	—	+	+	+	—	+	+	+	+
Blood vessel proliferation	—	+	+	+	+	+	+	+	+
Diagnosis‡	DNF	DF	U	DNF	U	JLI	DNF	DF	DNF

* —, absent; +, present; F, focal; D, diffuse.

† G, giant cells; P, plasma cells; E, eosinophilic granulocytes.

‡ DNF, dense non-follicular infiltrates; DF, dense follicular infiltrates; U, uncertain diagnosis; JLI, Jessner's lymphocytic infiltrate.

perfectly benign clinical course for 72 and 165 months, respectively (see Table 1), a definite benign diagnosis could not be made on routine histology.

The skin biopsies from the remaining patient (No. 6) diagnosed as Jessner's lymphocytic infiltrate on clinical criteria, also demonstrated histological changes consistent with this diagnosis. The biopsies from this patient differed from those of the other patients in several respects. First, the infiltrate was monomorphic being predominantly composed of lymphocytes. Second, the infiltrate demonstrated patchy involvement of appendages and of epidermis in the form of single cell exocytosis; and third, the pattern of infiltration was focal in both the upper and lower layers of the dermis. The cells did not demonstrate any cytological atypia. Neither Pautrier microabscesses, necrosis, vascular involvement nor degeneration of basal keratinocytes were identified.

Immunological analysis of skin biopsies

The data from immunoenzymatic labelling of frozen sections of skin biopsies with a panel of monoclonal antibodies are summarized in Table 4. Three of four patients with lymphocytoma

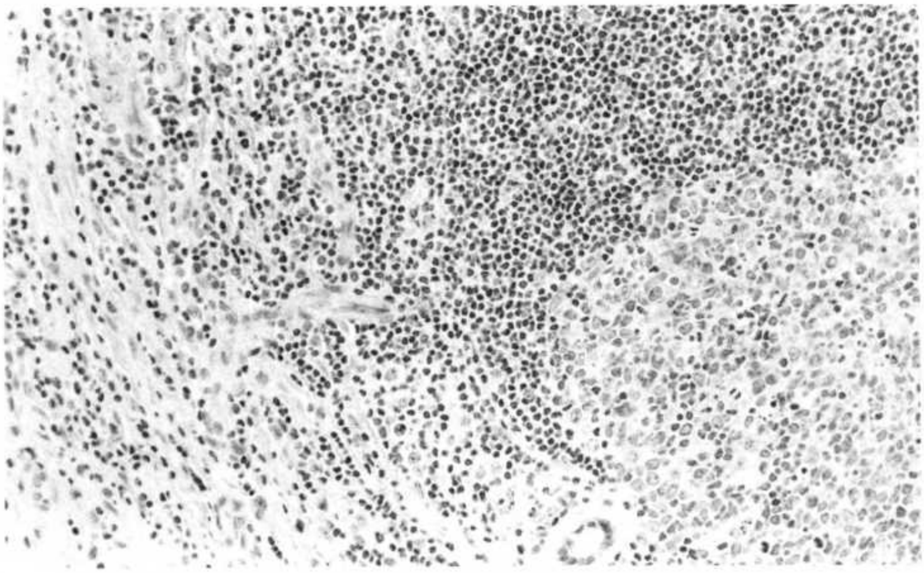

FIGURE 1. Germinal centre formation in the dermal infiltrate from benign cutaneous lymphoplasia (H & E, × 300).

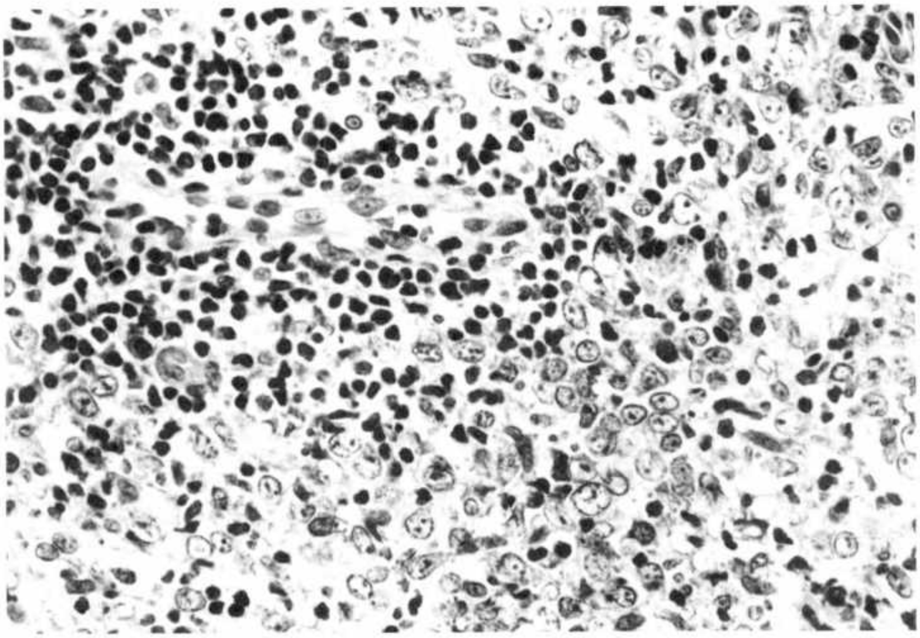

FIGURE 2. Cytological atypia in lymphoid cells from clinically benign lymphocytoma cutis (H & E, × 450).

TABLE 4. Cellular constituents in skin infiltrates

Patient	Clinical diagnosis*	Histological diagnosis	Polyclonal B-cell clusters	Follicular dendritic cells	T-helper/ inducer cells	T-suppressor/ cytotoxic cells	HLA-DR positive dendritic dermal cells†
1	BL	Lymphocytoma	+	+	++‡	+‡	+
2	BL	Lymphoplasia	+	+	++	+	+
3	BL	Uncertain	+	—	++	+	+
4	BL	Lymphocytoma	+	—	++	+	+
5	BL	Uncertain	+	+	++	+	+
6	JLI	JLI	—	—	+	++	+
7	BL	Lymphocytoma	+	—	++	+	+
8	BL	Lymphoplasia	+	+	++	+	+
9	BL	Lymphocytoma	—	—	++	+	+

* BL, benign lymphocytoma or lymphoplasia; JLI, Jessner's lymphocytic infiltrate.

† Include Langerhans cells (OKT6$^+$, HLA-DR$^+$) and related dendritic cells (OKT6$^-$, HLA-DR$^+$).

‡ + + Indicate the predominant T-cell subset (helper/inducer or suppressor/cytotoxic).

cutis (Nos 1, 4 and 7), both patients with lymphoplasia (Nos 2 and 8) and both patients with benign clinical courses, but uncertain histological diagnoses (Nos. 3 and 5) demonstrated similar cellular constituents in their skin infiltrates. Biopsies from different areas of skin involvement in three of these patients (Nos 1, 4 and 5) also demonstrated that the cellular constituents in different lesions were indistinguishable. In all these patients the dermal infiltrate consisted of both B lymphocytes (B1$^+$, To15$^+$, HLA-DR$^+$, C3b receptor$^{+/-}$) and T lymphocytes (Leu1/Lyt2$^+$, Leu5/Lyt3$^+$) (Fig. 3). The majority of the B cells expressed IgM and very few cells reacted with anti-IgD antibodies. The B cells were polyclonal as judged from an estimated ratio of kappa : lambda light chain-positive B cells of 3 : 1–1 : 1. In four patients (Nos 1, 2, 5 and 8), the B-cell clusters contained follicular dendritic cells (R4/23$^+$, C3b receptor$^+$) (Fig. 3b). The B-cell clusters thereby closely resembled the B-cell follicles seen in peripheral lymph nodes. Immunoenzymatic staining thus confirmed the morphological impression of germinal centre formation in the biopsies from two patients (Nos 2 and 8), and permitted the identification of B-cell follicles in the dermal infiltrates from two patients without morphological evidence of follicular structures (Nos 1 and 5, see Table 3). The T cells expressed a mature phenotype (Leu4$^+$, OKT6$^-$) and demonstrated a relative predominance of T helper/inducer cells with an estimated ratio of T helper/inducer (Leu3$^+$) : T suppressor/cytotoxic cells (Leu2$^+$) from 4:1 to 2:1.

The biopsies from the remaining two patients (Nos 6 and 9) differed from those of the other patients by the absence of demonstrable B cells. One of these patients had a clinical and histological diagnosis of cutaneous lymphocytoma (No. 9), and this patient demonstrated predominance of T helper/inducer cells. The other patient suffered from Jessner's lymphocytic infiltrate (No. 6) and this patient differed from all the other patients by having a predominance of T-suppressor/cytoxic cells.

In all patients, subsets of the infiltrating T cells expressed HLA-DR, indicating a state of activation of the cells (Reinherz *et al.*, 1979). This state of activation was further supported by the identification of transferrin receptors (OKT9$^+$) on subsets of the T cells in seven patients (Nos 2–4 and 6–9) and the T10 antigen in four patients (Nos 5–8).

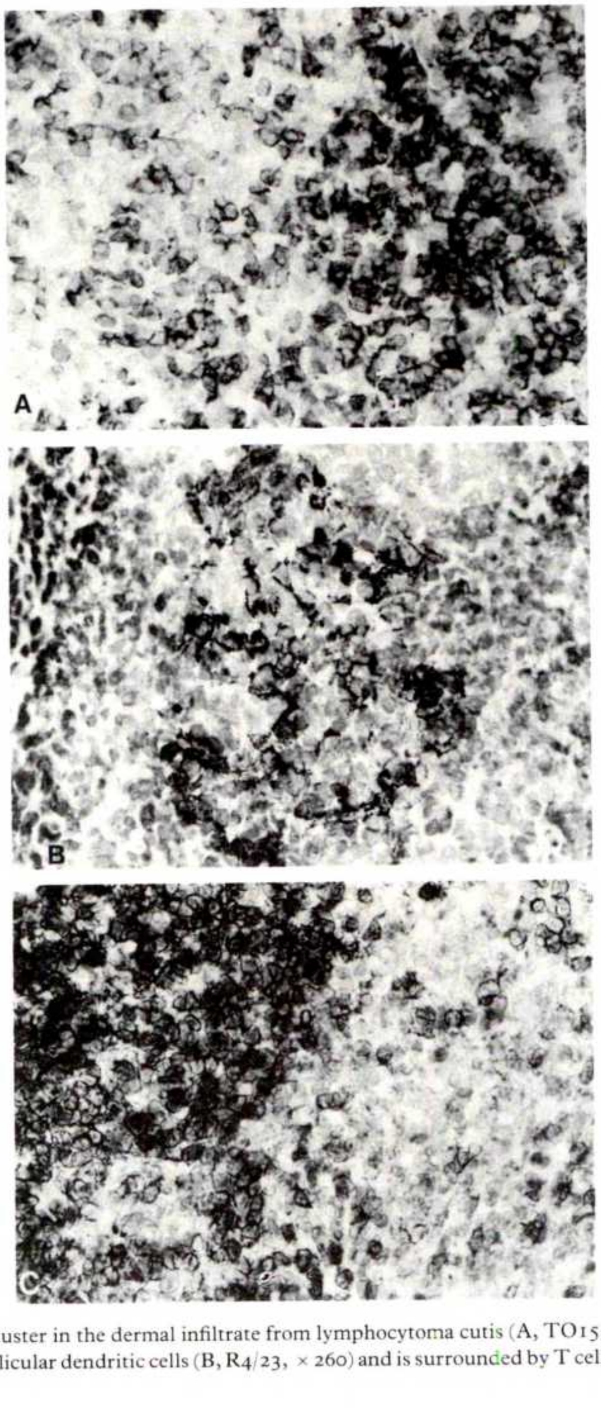

FIGURE 3. B-cell cluster in the dermal infiltrate from lymphocytoma cutis (A, TO15, × 260). The B-cell cluster contains follicular dendritic cells (B, R4/23, × 260) and is surrounded by T cells (C, Leu 4, × 260).

The dermal infiltrates from all patients also contained a considerable number of Langerhans cells and related HLA-DR positive dendritic dermal cells (HLA-DR$^+$, OKT6$^{+/-}$). The number of epidermal Langerhans cells (HLA-DR$^+$, OKT6$^+$) ranged from 5 to 27 in six patients (Nos 1, 3, 6–9) as compared with a mean number of Langerhans cells of twenty-six in biopsies of normal skin. The remaining three patients (Nos 2, 4 and 5) demonstrated a slightly increased number of epidermal Langerhans cells (39–58).

Monocytes (MO2$^+$) and natural killer cells (Leu7$^+$) were occasionally identified in the dermal infiltrate in all the biopsies examined. However, these cells constituted less than 1% of the infiltrating cells.

DISCUSSION

In this study, infiltrating cells in skin biopsies from nine patients with a histological and/or clinical diagnosis of benign lymphocytic skin infiltrate (lymphocytoma, lymphoplasia, Jessner's lymphocytic infiltrate) have been characterized by immunoenzymatic labelling of frozen sections with a panel of monoclonal antibodies against lymphocytes and accessory cells. Two patients with atypical cells but benign clinical course were included in the study, and it is well known that benign lymphocytic skin infiltrates may demonstrate considerable cytological atypia of the infiltrating cells (Clark *et al.*, 1974; Evans *et al.*, 1979; Lange Wantzin *et al.*, 1982; MacDonald, 1982). The immunological data further substantiated that these two patients did suffer from benign lymphocytic skin infiltrates, since their cellular constituents in skin biopsies were similar to those of other patients with lymphocytoma or lymphoplasia.

The most significant immunological finding was the demonstration of a mixed infiltrate of T cells, Langerhans cells, related HLA-DR positive dendritic, dermal cells and clusters of polyclonal B cells in the biopsies from all but one patient with lymphocytoma cutis or cutaneous lymphoplasia. Furthermore, in four of these patients, the B-cell clusters contained B-cell accessory follicular dendritic cells and thereby closely resembled the B-cell follicles seen in peripheral lymphoid tissue (Naiem *et al.*, 1983). These data extend the results obtained by examination of cell suspensions with rosetting techniques and less extensive panels of antibodies (Knowles *et al.*, 1982) and are clearly indicative of a benign diagnosis. Although quantitative immunological criteria for the discrimination between benign (polyclonal) and malignant (monoclonal) B-cell infiltrations of the skin are not yet available, our data differ markedly from results obtained by examination of skin lymphomas of B-cell origin, since these demonstrate preponderance of B cells restricted to synthesis of either kappa- or lambda-light chain immunoglobulins (Willemze *et al.*, 1983a). Malignant T-cell infiltrations also differ from the present biopsies, and demonstrate absence of B cells and a more pronounced predominance of T-helper/inducer cells and/or aberrant T-cell phenotypes (Willemze *et al.*, 1983b). The demonstration of similar cellular constituents in lymphocytoma cutis, cutaneous lymphoplasia (this study), benign hyperplastic lymph nodes (Ralfkiær *et al.*, 1984a), and some cases of patch tests (Ralfkiær & Lange Wantzin, 1984) further substantiates the benign nature of the infiltrating cells in the present study. This also supports the concept that lymphocytoma and lymphoplasia represent exaggerated immune responses (Knowles *et al.*, 1982) and indicates that immune reactions in the skin may follow the same patterns as those seen in lymphoid organs. In particular, the identification of B-cell accessory follicular dendritic cells in skin infiltrates from lymphocytoma and lymphoplasia extends our previous study of patch tests (Ralfkiær & Lange Wantzin, 1984), and suggests an involvement of B cells in certain immune reactions of the skin, and/or an involvement of the skin in the generation and expansion of memory B cells.

The close immunological resemblance between lymphocytoma cutis and cutaneous lymphoplasia indicates that these disorders are basically similar. In particular, our data emphasize that the formation of germinal centers and/or follicular structures cannot be used as a discriminating criterion (From, 1979).

The absence of detectable B cells in the skin infiltrates from one patient diagnosed as lymphocytoma cutis, and one patient diagnosed as Jessner's lymphocytic infiltrate may reflect sampling error, since B cells are distributed in clusters in the dermal infiltrate. Alternatively, benign lymphocytic skin infiltrates may demonstrate a spectrum of immune reactions and/or encompass different disease entities. The latter possibility is particularly pertinent to Jessner's lymphocytic infiltrate—which many authors consider to be a separate entity (Clark *et al.*, 1974; From, 1979; Lange Wantzin *et al.*, 1982).

In a previous study, the suggestion was made that C3 complement receptors are restricted to benign lymphocytic skin infiltrates as determined by adherence of complement-coated erythrocytes (EAC-rosettes) to tissue sections (Burg *et al.*, 1977). Since the EAC-rosetting cells localized in central areas of follicular structures, C3 complement receptors on follicular dendritic cells probably accounted for the tissue adherence pattern observed (Gerdes & Stein, 1982; Ralfkiær *et al.*, 1984b). It should, however, be noted that follicular dendritic cells have been identified in the dermal infiltrates from malignant B-cell lymphomas by histochemical criteria (Willemze *et al.*, 1983a). Our own preliminary data from immunohistological staining of malignant skin lymphomas of B-cell origin have confirmed this finding (E. Ralfkiær, unpublished data). This strongly indicates that the expression of C3 complement receptors is not specific for benign, as opposed to malignant, lymphocytic skin infiltrates.

The present data demonstrate that *in situ* immunological analysis is extremely valuable for the identification of cells (e.g., B cells, follicular dendritic cells) which cannot be distinguished by morphological criteria. The method may thereby provide valuable information concerning the nature and origin of the infiltrating cells in benign lymphocytic skin infiltrates and may also assist in establishing a definite diagnosis in cases with atypical cytological features.

ACKNOWLEDGMENTS

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Cellular retinol- and retinoic acid-binding proteins in the epidermis and dermis of normal human skin

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SUMMARY

The distribution of cellular retinol- and retinoic acid-binding proteins (CRBP and CRABP) in normal human epidermis and dermis was examined by gel filtration. We showed that CRBP is entirely bound to a lipid–protein aggregate and its free form is obtained through delipidation. The type of homogenization procedure appeared to be important for the recovery of CRBP in human skin. The level of CRABP was about 3·1 pmol/mg protein in the epidermis whereas it was only sporadically detectable in the dermis. In contrast, CRBP was found in both tissues at a concentration of about 1 pmol/mg protein. The difference in CRABP concentrations between epidermis and dermis might have biological and therapeutic implications. Dissociation constants (K_d) of CRABP and CRBP were respectively $2{\cdot}2 \times 10^{-7}$ M and $2{\cdot}51 \times 10^{-7}$ M. This method will facilitate the study of CRABP and CRBP in retinoid- responsive dermatoses and enable us to relate the therapeutic effects of retinoids to the levels of cellular retinoid-binding proteins in the skin.

Two intracellular retinoid-binding proteins are thought to be involved in the mode of action of retinoids at the molecular level: cellular retinol-binding protein (CRBP) (Bashor, Toft & Chytil, 1973; Ong & Chytil, 1978a; Ross, Takahashi & Goodman, 1978; Saari, Futterman & Bredberg, 1978) and cellular retinoic acid-binding protein (CRABP) (Saari *et al.*, 1978; Ong & Chytil, 1978b; Ross *et al.*, 1980). They have a similar molecular weight of about 14,000 daltons but CRBP specifically binds retinol whereas CRABP binds retinoic acid (Bashor *et al.*, 1973; Ong & Chytil, 1975; Sani & Hill, 1974).

Although both CRBP and CRABP have been demonstrated in many organs, including the skin of rat and chicken (Sani & Banerjee, 1978; Ong, Crow & Chytil, 1982), no data about human skin are presently available. There are two important reasons for studying CRBP and CRABP in human skin: (i) both can be postulated as being specific receptors for the synthetic retinoids now widely used as therapeutic agents in dermatology (Pawson *et al.*, 1982); and (ii) both might be

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altered in retinoid-responsive skin diseases (Peck, 1981). Since the two compartments of the skin, dermis and epidermis, are not always similarly involved in skin diseases and do not seem to respond in the same way to systemically administered retinoids, it is important to study CRABP and CRBP separately in both epidermis and dermis.

METHODS

The all-*trans*-[11,12-^{3}H]retinoic acid (30 Ci/mmol) used was a gift from F.Hoffmann-La Roche (CH-4000 Basle, Switzerland). All-*trans*-[15-^{3}H]retinol (14·3 Ci/mmol) and Biofluor were purchased from NEN (F.R.G.). All-*trans*-retinoic acid, retinol and DL-α-tocopherol were obtained from Sigma. Sephadex G75 superfine, Blue Dextran 2000 and ribonuclease A were purchased from Pharmacia (Uppsala, Sweden). All other chemicals were of analytical grade. Water was double-distilled. The Polytron homogenizer was obtained from Kinematica (CH-6010 Lucerne, Switzerland). The radioactivity was counted in a Nuclear Chicago Isocap/300 liquid scintillation spectrophotometer.

Cytosolic fraction

Skin specimens were obtained from the abdomen of 29- to 52-year-old healthy women undergoing plastic surgery. Defatted specimens of skin were stored frozen at −70°C until used. Epidermis was separated from dermis by heat (52°C, 1 min) in saline (Michaelson *et al.*, 1980). Specimens were checked to ensure that the separation had occurred between epidermis and dermis. Freeze-dried epidermis representing an aliquot of about 20 mg of dry tissue or 50 mg of dry dermis was homogenized in 800 μl of 50 mM Tris/HCl, buffer pH 7·4, containing NaCl (25 mM) and EDTA (2·5 mM) using a Polytron PT-20 homogenizer, followed by centrifugation at 10,000 ***g*** for 5 min. A Potter Elvehjen homogenizer was used for studying CRBP bound to the aggregate. The resulting supernatant was then centrifuged at 100,000 ***g*** for 60 min to obtain the cytosolic fraction which was used immediately for assays. Protein concentration was estimated by the Lowry method (Lowry *et al.*, 1951). Lipids were extracted from the cytosol fraction prior to studies of CRBP bound to the aggregate by vigorous mixing with two volumes of diethyl ether at 0°C. The organic layer was rejected after centrifugation of the mixture for 5 min at 10,000 ***g***.

Incubation and gel filtration

All the following steps were conducted at 4°C and under red light. The alcoholic retinoid solutions all contained α-tocopherol (1 mg/ml) as antioxidant. [^{3}H]retinol or [^{3}H]retinoic acid, to give a final concentration in the incubation mixture of 60 nM and 10 nM, respectively (non-saturating conditions) was deposited in a glass microtube and ethanol was evaporated with a stream of N_2. An aliquot of cytosolic fraction (300 μg of protein) in a final volume of 100 μl was added and incubated. Preliminary experiments showed that incubation of cytosol protein with [^{3}H]retinoids at 4°C for 16 hours was sufficient for binding equilibrium to be established. For binding competition studies an excess of 200-fold of unlabelled ligand was added in the same manner as for the isotope. After incubation, each sample was subjected to gel filtration on a column of Sephadex G75 (0·8 × 15 cm) equilibrated with the extraction buffer containing sodium azide (10 mM). The column was calibrated with ribonuclease A (13,700 daltons) and its void volume was determined with Blue Dextran 2000. Before application of each sample the column was loaded with 1 ml of 3% bovine serum albumin dissolved in column buffer and eluted with 80 ml of the buffer. Fractions of 300 μl were collected at a flow rate of 6 ml/h and mixed with 8 ml of Biofluor before being counted (counting efficiency <45%). CRABP or

CRBP were identified as the radioactive peaks on gel filtration with an elution volume corresponding to that of ribonuclease A. Each binding activity was expressed as fmol of the ligand specifically bound per mg of protein or in pmol per g of lyophilized tissues, by calculation of the net radioactive count with or without unlabelled competitors.

Equilibrium binding affinity

Preliminary evaluation of retinoic acid or retinol equilibrium binding affinity was performed using the method of Scatchard (Scatchard, 1949). Aliquots of cytosol (0·31 mg) were incubated with [^{3}H]retinoic acid (5–800 nM) in the presence or absence of a 200-fold excess of retinoic acid. The specific saturable binding to the CRAPB peak was determined on Sephadex G75 elution profile and plotted. For the analysis of specific saturable [^{3}H]retinol binding, aliquots of defatted cytosol (0·25 mg) were incubated with [^{3}H]retinol (20–1000 nM) in the presence or absence of a 200-fold excess of retinol. Specific saturable binding to the CRBP peak was determined as for CRABP. The reproducibility of the equilibrium binding affinity points was checked twice.

RESULTS

CRABP in human epidermis and dermis

The elution profile of the cytosolic fraction of human epidermis incubated with [^{3}H]retinoic acid is shown in Fig. 1. The first radioactive peak eluted from the gel corresponded to the lipid–protein aggregate and to albumin. The second peak which was co-eluted with ribonuclease was the CRABP and the third one was the free ligand. When cytosol was incubated in the presence of 200-fold excess of non-radioactive retinoic acid, the radioactivity in the second peak was displaced. On the other hand the radioactive peak of CRABP was not displaced by an excess of non-labelled retinol (data not shown), confirming the specificity of the binding site.

The reproducibility of the method was tested using four identical lyophilized epidermis samples from the same person. The variation of the concentration of CRABP sites, expressed in

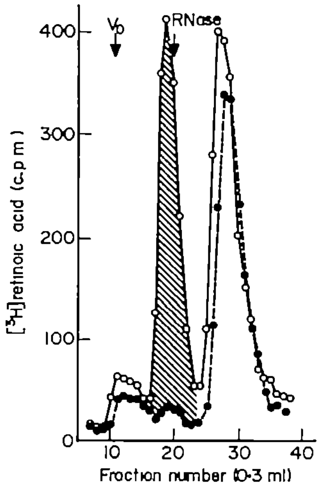

FIGURE 1. Gel filtration analysis on Sephadex G75 of [^{3}H]retinoic acid binding in human epidermis cytosol. Protein (0 49 mg) was incubated with 10 nM [^{3}H]retinoic acid (○——○) and the same experiment was repeated with a 200-fold excess of unlabelled retinoic acid (●——●).

TABLE 1. [^{3}H]Retinoic acid binding activities (CRABP) in the skin. The values correspond to non-saturating conditions

			CRABP	
		mg protein/g tissue*	fmol/mg protein	pmol/g tissue*
Epidermis	($n=7$)	99·9 ± 13·4	140·6 ± 31·9	13·7 ± 3·6
Dermis	($n=5$)	46·2 ± 12·2	26 (range 0–57)	1·6 (range 0–2·8

n = number of healthy subjects.
* Lyophilized tissues.

fmol/mg protein, was about 20%. The variations of the level of CRABP in human epidermis and dermis as measured in non-saturating conditions are shown in Table 1. It was demonstrated that though epidermis always contains CRABP (140·6 ± 31·9 fmol/mg protein) this was only sporadically measurable in dermis.

CRBP in human epidermis and dermis

When the cytosolic fraction of epidermis was incubated with [^{3}H]retinol in the presence or absence of unlabelled retinol and submitted to gel filtration, the elution profiles shown in Fig. 2 were obtained. When the homogenization of the tissue was done with the Potter Elvehjem, only trace amounts of CRBP appeared at the same elution volume as ribonuclease A. However, the first radioactive peak was endowed with specific binding activity since the radioactive peak was partially displaced by an 200-fold excess of retinol (cf. Fig. 2a). In contrast, when the homogenization of the same tissue was done with the Polytron we obtained the elution profile shown in Fig. 2b. The first peak was diminished and the peak of free CRBP was more pronounced. Moreover, when the cytosolic fraction was delipidated with diethyl ether, the first radioactive peak completely disappeared and the CRBP was obtained entirely in its free form (Fig. 2c). The competitive displacements calculated for conditions (a) and (b) (Fig. 2) quantitatively corresponded to that of (c). A 200-fold excess of retinoic acid did not displace the radioactive peaks of [^{3}H]retinol. This fact ruled out contamination by RBP from serum because retinoic acid has *in vitro* almost the same affinity for RBP as retinol. (Cogan *et al.*, 1976). The concentration of CRBP sites was therefore equal to the total displacement of the different elution profiles in Fig. 2 for this non-saturation experimental condition. In the dermis, the CRBP was also bound to the lipid–protein aggregate and its release was dependent on the type of homogenization. CRBP was found in very similar concentrations in human epidermis and dermis (Table 2), with mean values of 234·1 ± 54·4 fmol/mg protein. As the dermis contains less solubilized protein than the epidermis, the values expressed in pmol/g of tissue are consequently lower.

Determination of binding constants of CRABP and CRBP

Figure 3 shows the Scatchard plots of the steady-state binding of [^{3}H]retinoic acid and [^{3}H]retinol with the cytosolic fraction of two different human epidermis specimens. The lines indicate a single class of binding sites for both CRABP and CRBP and the slopes reveal relatively low binding affinities with equilibrium dissociation constants of $K_d = 2{\cdot}2 \times 10^{-7}$M for CRABP and $K_d = 2{\cdot}51 \times 10^{-7}$ M for CRBP (mean of two experiments).

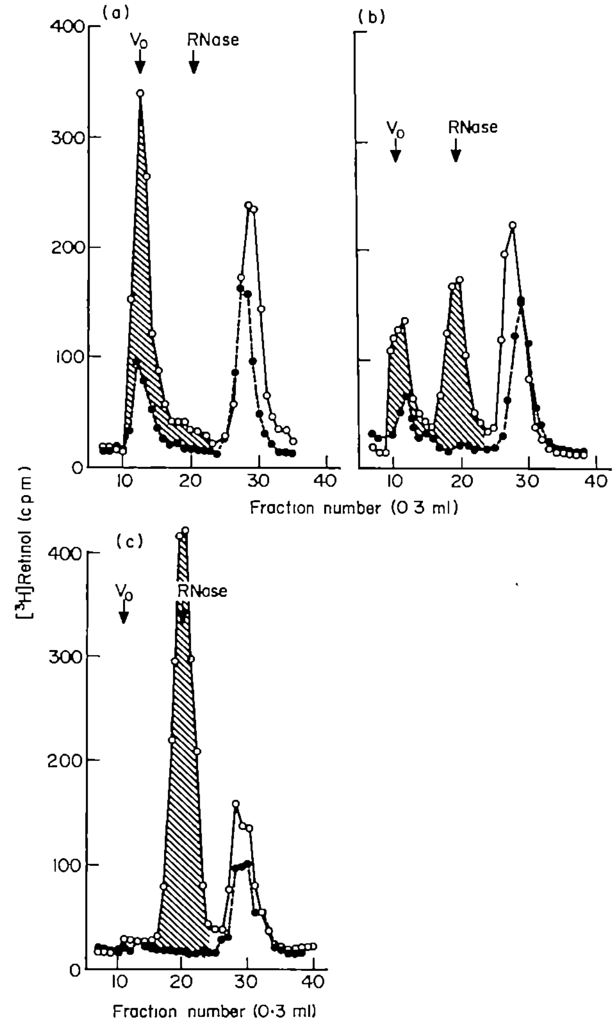

FIGURE 2. Gel filtration analysis on Sephadex G75 of [^{3}H]retinol binding in human epidermis cytosol. Three aliquots of protein were incubated with 50 nM [^{3}H]retinol (○——○) and the same experiment was repeated with 200-fold excess of unlabelled retinol (●——●). Homogenization of the tissue with Potter (a), with Polytron (b) and with Polytron followed with delipidation of the cytosol (c).

TABLE 2. [^{3}H]Retinol binding activities (CRBP) in the skin. The values correspond to non-saturating conditions

			CRBP	
		mg protein/g tissue*	fmol/mg protein	pmol/g tissue*
Epidermis	(n = 8)	97·6 ± 12·6	234·1 ± 54·4	23·9 ± 5·3
Dermis	(n = 3)	37·6 ± 6·6	272·4 ± 34·2	9·3 ± 2·7

n = number of healthy subjects.
* Lyophilized tissues.

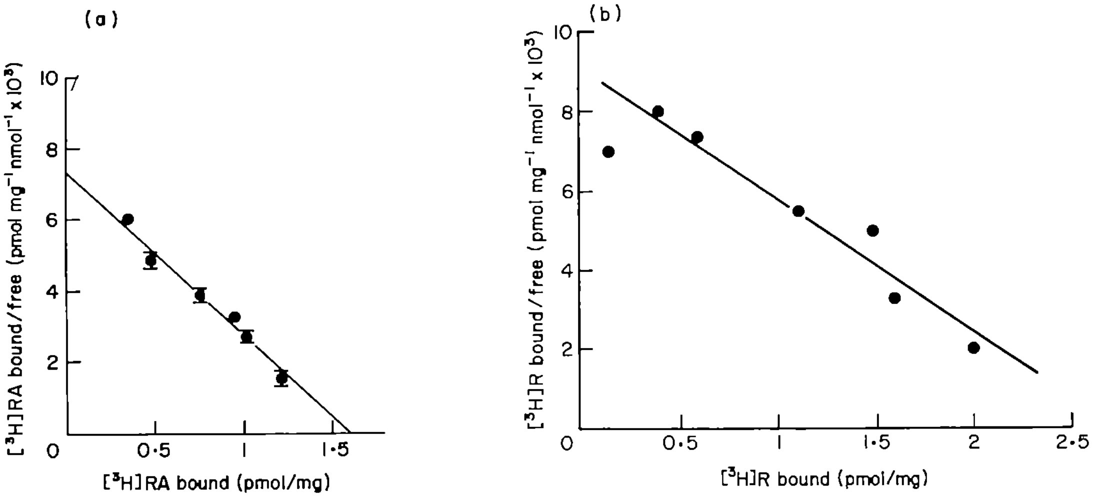

FIGURE 3. Scatchard analysis of specific saturable [^{3}H]retinoic acid binding to CRABP (a) and specific saturable [^{3}H]retinol binding to CRBP (b) from human epidermis cytosol. Conditions were as described under Methods. Each Scatchard analysis point represents two determinations.

DISCUSSION

This is the first attempt to detect CRABP and CRBP differentially in both normal human epidermis and dermis. The results show that these two binding proteins can be independently measured in human epidermis and dermis. The reproducibility of the method is ± 20% and its sensitivity is about 10 fmol.

This gel filtration technique appears to be more convenient than sucrose gradient centrifugation, as it does not require a long ultracentrifugation (Muto & Omori, 1981). Moreover, gel filtration permits the separation of the lipidic contaminants without acid treatment (pH 5) (Ong & Chytil, 1976) of the cytosol which might precipitate the binding proteins whose pI is very near to 5. Binding to this contaminant is often considered to be non-specific because of the relatively low proportion of radioactivity displaced by unlabelled ligand (Adachi *et al.*, 1981).

We demonstrated that epidermal and dermal CRBP are completely bound to the lipid–protein aggregate. We also established that, depending on the homogenization method, the CRBP is only partially released from the aggregate, whereas after cytosol delipidation, it is found entirely in the free form. Goodman's group (Sklan *et al.*, 1982) has demonstrated that the liver cytosol CRBP was associated with an aggregate of high molecular weight composed of lipids and proteins. Moreover, they showed that immunoreactive CRBP was detectable only if the samples were pretreated with detergents (Adachi *et al.*, 1981). Our results confirm these observations about the CRBP aggregate binding and extend its applicability to two human skin compartments.

The use of detergents (CHAPS, Triton X-100, Lubrol) in concentrations of 0·5 to 1% in our incubation or for the elution buffer of the columns did not permit us to separate CRBP from the aggregate. Detergents dramatically increased the non-specific affinity of retinoids for the aggregate. We think that the CRBP complexed to the aggregate is most probably the native form of the binding protein and not an extraction artifact. When homogenization is done with a Potter (less destructive) the CRBP remains bound to the aggregate, whereas use of a more violent homogenization method (e.g. Polytron with or without delipidation) causes release of CRBP.

The CRABP does not behave similarly; it might be less hydrophobic than CRBP. It is possible that these properties of CRBP indicate that it is membrane bound. CRBP might be the vector of the retinyl hydrolase product (retinol). Retinyl hydrolase is an enzyme found in the lipid–protein aggregate of the liver cytosol.

On the other hand, CRBP could play the part of second relay after the serum RBP in the vitamin A transport within the cell. Although serum RBP (21,000 daltons) binds *in vitro* retinoic acid and retinol (Cogan *et al.*, 1976), no serum RBP binding activity has been observed in the elution profile of the column between the first and second radioactive peaks.

Since it has been suggested, from radial immunodiffusion (Törma & Vahlquist, 1983) and immunohistological observations (Forsum *et al.*, 1977; Sundelin *et al.*, 1983), that RBP was detectable in the epidermis, it might be that epidermis contains RBP in immunoreactive form but that RBP has lost its affinity for retinoid. In fact we did detect RBP by immunoelectrophoresis in the supernatants devoid of RBP-binding activity (unpublished results).

In our experimental conditions CRABP was always present in the epidermis, whereas it was only sporadically found in the dermis (mean values were five times lower). The value of CRABP in the epidermis was 3·1 ± 0·7 pmol/mg protein, as calculated with a K_d of $2{\cdot}2 \times 10^{-7}$ M and the value given in Table 1.

In contrast, CRBP was found at the same concentration in epidermis and dermis. Its value, calculated using a K_d of $2{\cdot}51 \times 10^{-7}$ M and the value given in Table 2, is found to be 1·0 ± 0·23 pmol/mg protein. CRABP but not CRBP is detectable in human fibroblast culture (Lacroix, Anderson & Lippman, 1981). It is possible that the expression of the intracellular binding proteins is different when measured *in vitro* and *in vivo*. The method of protein extraction might contribute to the differences observed. The dissociation constants of the two intracellular binding proteins are relatively low but of the same order as that of serum RBP ($K_d = 1{\cdot}9 \times 10^{-7}$ M), as determined by fluorometric titration (Cogan *et al.*, 1976) and that of CRABP from human mammary gland ($K_d = 2{\cdot}5 \times 10^{-7}$ M) as determined by polyacrylamide disc gel electrophoresis (Kung *et al.*, 1980).

Although the mechanisms of action of retinol and retinoic acid are unknown, increasing evidence suggests that their effects are mediated in part by CRABP and CRBP (Pawson *et al.*, 1982). Our results suggest that epidermis is a retinoic acid- and retinol-sensitive tissue whereas the dermis might be more sensitive to retinol than to retinoic acid. This is in accordance with the observation that in animals CRABP was found to be present in the epithelial cells but not in the connective tissue of skin (Sani & Corbett, 1977).

Retinoids used therapeutically have a dramatic effect on normal and pathological skin. The most obvious change is desquamation (Peck, 1981) which reflects a modification of epidermal homeostasis. Biological effects on the dermis are not well documented *in vivo*. The differences in CRABP concentrations between epidermis and dermis might be relevant to these biological and therapeutic effects.

At any rate the methods described here will allow us to study both CRBP and CRABP in retinoid-responsive skin diseases and to relate the therapeutic effects of these potent drugs to the amount of cellular retinoid binding proteins in the skin.

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The semi-quantitative distribution of T4 and T6 surface antigens on human Langerhans cells

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SUMMARY

We have used indirect immunogold electron microscopy to compare the respective density of cell membrane determinants revealed by OKT6 and OKT4 monoclonal antibodies on normal human Langerhans cells (LC): 12·9 ± 3·5 gold granules were noted per cell section on OKT4-positive LC whereas 236·8 ± 23·5 granules were counted per cell section on OKT6-reactive cells. These results confirm that human LC react with OKT4 antibody and they demonstrate a marked quantitative difference on LC surface between the antigenic determinants recognized by OKT6 and OKT4 antibodies.

OKT6 monoclonal antibody (MCA) which reacts with a surface antigen of cortical thymocytes (Reinherz *et al.*, 1980) has been shown to bind specifically to human Langerhans cells (LC) (Fithian *et al.*, 1981; Murphy *et al.*, 1981a,b) in the epidermis. This OKT6 MCA has been largely used to identify LC in skin sections (Holden *et al.*, 1982; Murphy *et al.*, 1981a,b) and in epidermal cell suspensions (Chu *et al.*, 1982; Czernielewski *et al.*, 1983).

Moreover, the reactivity of normal and pathological human LC with MCA specific for T4 antigens usually shared by circulating helper T lymphocytes (Reinherz *et al.*, 1979) has recently been reported (Wood, Warner & Warnke, 1983; Murphy *et al.*, 1983).

Using the immunoperoxidase technique Murphy *et al.* (1983) studied the reactivity of normal human LC and tumour cells of histiocytosis X (HX cells) with various MCA directed against T3, T4, T6 and T8 antigens (Reinherz *et al.*, 1980). In their study, LC reacted only with anti-T6 MCA (OKT6) whereas HX cells showed a reactivity with both anti-T6 and anti-T4 MCA (respectively, OKT6 and OKT4 MCA). On HX cells, OKT4 produced focal membrane staining which was strikingly less intense on immunoelectron microscopy than that of OKT6. Lastly, normal LC and HX cells did not react with OKT3 and OKT8 MCA.

On the other hand, Wood *et al.* (1983) studied the reactivity of various MCA (anti-Leu 3a/3b, OKT4 and anti-T4 from Coulter Electronics, U.S.A.) with LC, HX cells and other cells of the

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mononuclear phagocyte lineage using the indirect immunofluorescence technique on tissue sections or cell suspensions. Microscopical observations and fluorescence-activated cell sorter analysis showed reactivity of LC and HX cells with OKT4 and anti-T4 MCA which was less intense than that noted with OKT6.

In the present study the OKT6 or OKT4 MCA bound to human LC were revealed by the use of colloidal gold conjugate (Horisberger, 1979) and the densities of T6 and T4 antigenic sites were compared by quantification of the gold granules bound to the cell surfaces.

METHODS

Preparation of epidermal cell suspensions

Single cell suspensions were prepared from freshly removed normal human skin (plastic surgery—breast and abdomen). Briefly, trimmed skin was split-cut with a keratotome set at 0·1 mm. The resulting slices were treated for 60 min at 37°C with 0·3% trypsin (Difco, Detroit, U.S.A.) in buffer (0·68% NaCl, 0·04% KCl, 0·1% glucose, 0·22% $NaHCO_3$, pH 7·3). The epidermis was detached from the dermis with fine forceps. Isolated epidermal samples were pooled and dissociated in Minimum Essential Medium (MEM, Gibco, Glasgow, U.K.) supplemented with 20% fetal calf serum (FCS). The collected cells were resuspended in RPMI 1640 (Gibco, Glasgow, U.K.) supplemented with 15% heat-inactivated AB human serum, 20 mmol/l HEPES buffer, 2 mmol/l L-glutamine and antibiotics, then counted and the viability estimated by trypan blue exclusion. Viability, after trypsinization, was 85% or higher and after 18 h maintenance exceeded 75%.

Enrichment of epidermal cell suspensions

LC enrichment was obtained by Ficoll-Hypaque (Pharmacia France SA, Le Chesnay, France) sedimentation. Dispersed skin cells (4×10^6 cells/ml) in TC 199 (Gibco, Glasgow, U.K.) supplemented with 20% FCS were layered on the gradient and centrifuged for 30 min at 400 ***g*** at 4°C. The lymphoid-like cells at the interface were washed and resuspended in phosphate-buffered saline (PBS) pH 7·2. The suspension usually contained 10–20% of OKT6-positive cells.

Monoclonal antibodies (MCA)

OKT6 and OKT4 MCA (Ortho Pharmaceutical Corp. Raritan N.J., U.S.A.) were used at the same dilution (1:5 in PBS).

In all experiments, control cell suspensions were incubated with normal mouse serum (NMS) diluted 1:20 or PBS in the first step of the immunogold labelling procedure instead of OKT6 and OKT4 MCA.

Immunogold labelling procedure

Briefly, the details of the technique have been previously reported (Schmitt *et al*., 1983; Schmitt *et al*., 1984), EC suspensions were fixed by 3% paraformaledehyde in PBS. After washing, OKT6 or OKT4 MCA were applied to specimen cells for 30 min at 37°C at a dilution of 1:5. The murine antibodies, fixed on the cell surface, were identified by the use of goat anti-mouse immunoglobulin labelled with gold granules of 40 nm size (GAM 40, Janssen, Pharmaceutica, Beerse, Belgium) applied at a dilution 1:2 for 1 h at 37°C. After washing with PBS, the cells were post-fixed for 20 min with 2% glutaraldehyde and 1% osmium tetroxide and embedded in epoxy medium. Ultrathin sections were examined after post-staining with lead citrate and uranyl acetate with a Philips EM 300 Electron microscope (Centre de Microscopie Electronique, CMEABG, Université Lyon I, Villeurbanne).

Quantitative analysis of T4 and T6 reactivity of LC

The cells analysed were those sectioned near their mid-plane to minimize tangential membrane sections and to provide a uniform morphological comparison between cells. In each group of experiments (OKT6 labelling or OKT4 labelling) we have examined ten LC sections. Under electron microscope, the number of gold particles fixed along the circumference of the cell membrane was counted to determine the relative extent of surface labelling. When gold granule aggregates were bound to the cell membrane, only the particles in close contact with the membrane were considered.

Labelling data are reported as mean number of gold granules fixed per LC section.

RESULTS

Ultrastructural aspects of LC surface labelling using immunogold conjugates

The labelled cells were identified by the presence of variable numbers of electron-dense gold particles scattered along the cell surface. The labelling appeared as single particles or small clusters of granules in direct apposition with the plasma membrane. No gold particles were

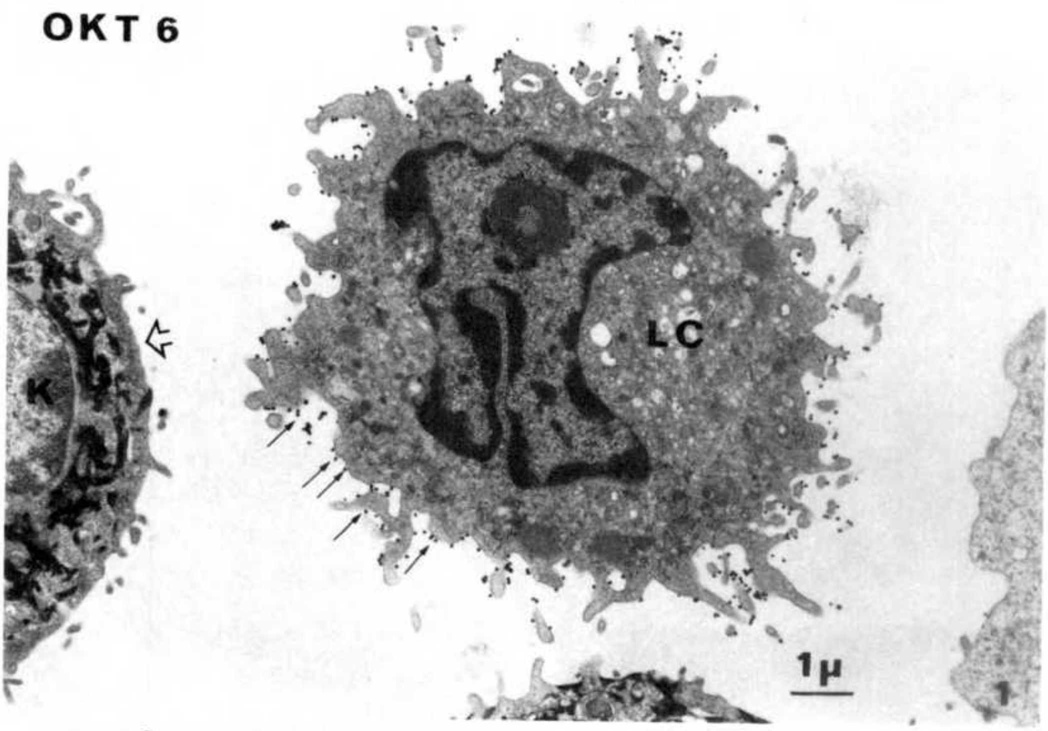

FIGURE 1. Immunogold labelling of a Langerhans cell (LC) for OKT6 MCA positivity. The arrows indicate the punctate pattern of the colloidal gold particles along the cell membrane. Note the absence of granules along the keratinocyte (K) surface.

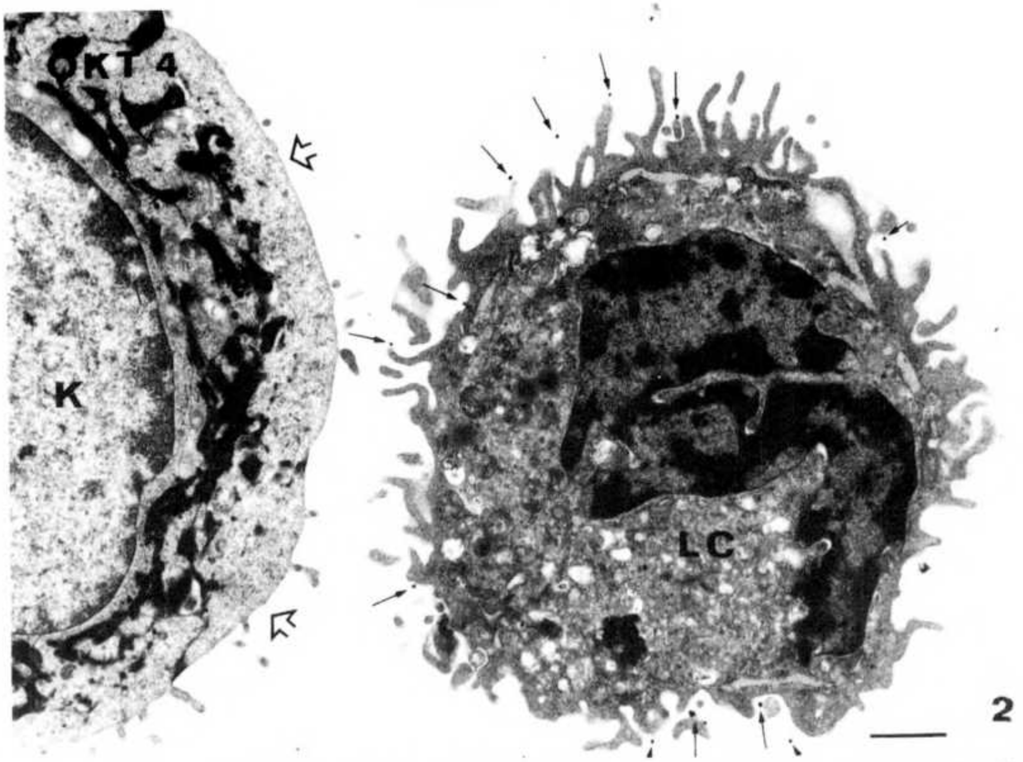

FIGURE 2. Immunogold labelling of a Langerhans cell (LC) for OKT4 positivity. The arrows visualize all the gold granules bound to the cell surface. Note the absence of keratinocyte (K) reactivity.

observed within the cytoplasm of these cells. Keratinocytes and melanocytes were negative with the two MCA used in this study.

Reactivity of LC with OKT6 MCA (Figs 1 and 2)
All the cells expressing T6 antigen revealed by the presence of gold granules were noted to contain cytoplasmic Birbeck granules, characteristic of LC. All T6-positive cells showed a strong reactivity with OKT6 as demonstrated by the large number of gold granules regularly bound to the cell surface.

Reactivity of LC with OKT4 MCA (Figs 3 and 4)
OKT4-labelled cells were identified by the presence of the 40-nm gold granules fixed on the membrane. All OKT4-positive cells showed Birbeck granules and were thus considered as typical LC. The number of gold particles of T4-positive cells was regularly low. These particles were randomly dispersed along the cell surface.

Control specimens of the same LC enriched suspensions (PBS and NMS) were negative for the presence of gold particles.

Comparative estimation of T6 and T4 antigenic sites of human LC (Table 1)
The quantitative results are reported in Table 1 (mean number of bound gold granules with standard error for T6 and T4 positive cells calculated per cell section).

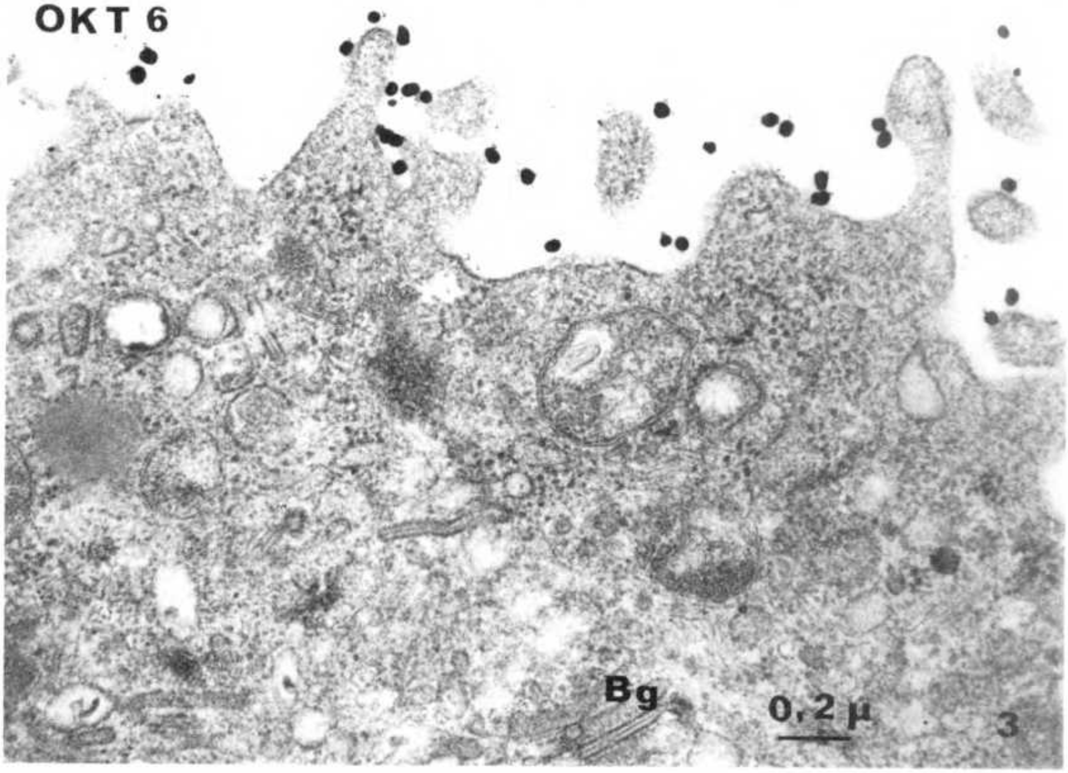

FIGURE 3. High magnification of the membrane labelling of a LC with OKT6 MCA. Bg: Birbeck granules.

When LC were labelled with OKT6 MCA a mean of 236·8 ± 23·5 gold granules could be observed along the membrane of the cell section with gold particles of 40 nm in diameter. When LC were labelled with OKT4 (used at the same dilution with LC suspensions obtained from the same skin sample) a mean of 12·9 ± 3·5 gold granules could be observed on the surface of the cell section with particles of the same size.

These results clearly demonstrate different expressions of these two surface antigens according to the labelling intensities.

DISCUSSION

LC surface antigens can be detected by various techniques including immunofluorescence (Fithian *et al.*, 1981), immunoperoxidase in light (Murphy *et al.*, 1981a,b) or electron microscopy (Murphy *et al.*, 1981a,b; Chu *et al.*, 1982; Holden *et al.*, 1982; Murphy *et al.*, 1983; Czernielewski *et al.*, 1983). We have recently developed an ultrastructural immunogold labelling for better identification of LC surface antigens (Schmitt *et al.*, 1983; Schmitt *et al.*, 1984).

The binding of gold particle-conjugated antibody to membrane antigens is primarily determined by the size of the granule. In fact, because of steric hindrance, the accessibility of the gold granule-conjugated antibody to receptors depends upon the size of the gold particles

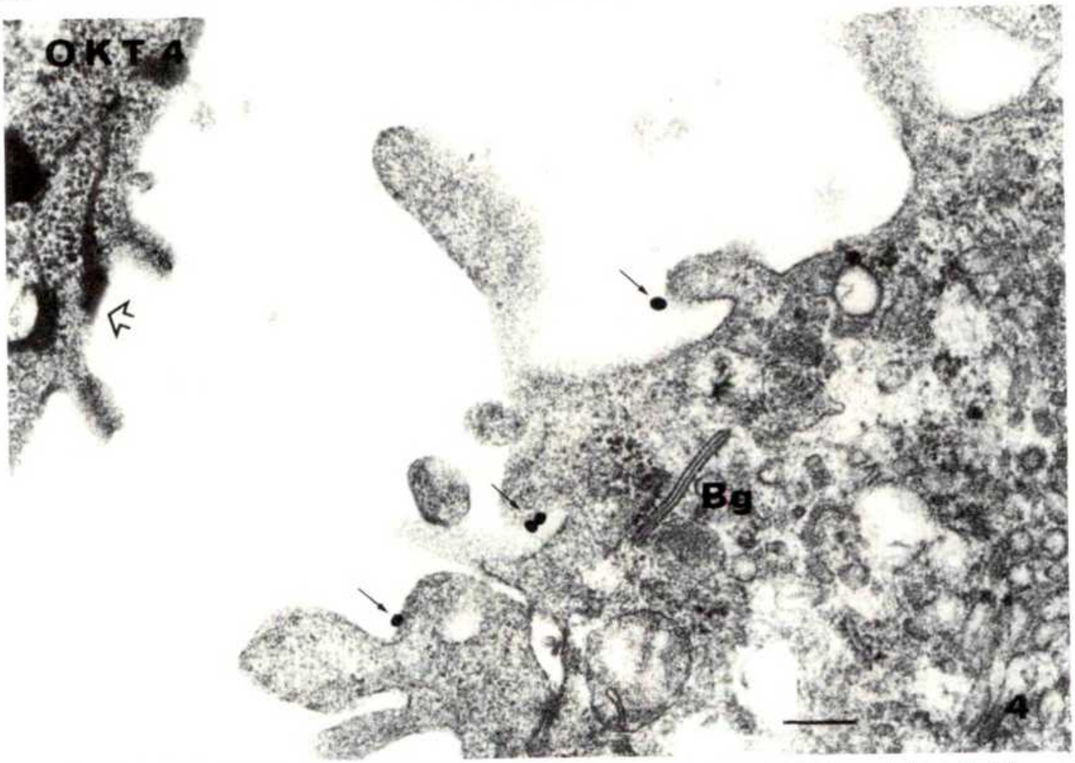

FIGURE 4. High magnification of the membrane labelling of a Birbeck granule (Bg) containing cell with OKT4 MCA. The arrows underline the 40 nm gold granules bound to the cell surface.

TABLE 1. Comparative quantification of gold particles fixed on the LC surface per cell section after labelling with OKT6 and OKT4 monoclonal antibodies

Monoclonal antibody	Number of LC sections considered	Mean number of gold granules fixed per LC section
OKT6	10*	236·8 ± 23·5†
OKT4	10	12·9 ± 3·5

* LC sections chosen among the midplane sections to minimize the error induced by tangential sections.

† Mean number of gold granules of 40 nm in diameter counted under electron-microscope; when aggregates of granules are present, only the gold granules in close contact with the cell membrane were considered.

(Horisberger & Tacchini-Vonlanthen, 1983). With particles of large diameter (40 nm) the number of granules fixed on the cell surface largely minimizes the real number of antigenic sites revealed by the immunological conjugate.

In our study, the comparison between the two mean numbers of granules only gives a semi-quantitative evaluation of the densities of T6 and T4 antigens by human LC.

The present results confirm the existence of T4 antigens on LC surface and a great difference in the densities of membrane determinants recognized by OKT6 and OKT4. These data are in accordance with the results of Wood *et al.* (1983) obtained by indirect immunofluorescence. The absence of reactivity of LC with OKT4 MCA observed by Murphy *et al.* (1983) using the immunoperoxidase technique could be explained by the difficulty of determining a clear-cut positivity with the enzymatic reaction product when the density of surface antigens is too low.

The existence of T6 antigens on the membrane of HX cells is now clearly established (Schuler *et al.*, 1983; Murphy *et al.*, 1983; Cambazard *et al.*, 1983; Rousseau-Merk *et al.*, 1983). The expression of T4 antigens by these tumour cells have been recently reported (Murphy *et al.*, 1983; Wood *et al.*, 1983). Immunoelectronmicroscopic investigations using the immunogold technique are in progress to compare the T4 antigen expression by LC and HX cells.

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A portable instrument for quantifying erythema induced by ultraviolet radiation

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SUMMARY

The design and performance of an instrument for quantifying ultraviolet-induced cutaneous erythema are discussed. If the instrument is used to record an 'erythema index' at a site on the skin before and after irradiation, the difference between those two readings is essentially related to an increase in vasodilation and is largely independent of the melanin content of the epidermis.

The grading of the degree of ultraviolet radiation-induced erythema was first performed by Hausser & Vahle (1927), who used red-coloured papers as visual standards of comparison. A similar technique whereby a set of red photographic filters was placed over erythematous areas was described by Berger, Urbach & Davies (1968).

The principle of operation of the instrument described here is based upon the technique of reflectance spectrophotometry and utilizes the fact that haemoglobin present in the blood vessels in the upper dermis is the main cutaneous chromophore of green light. The technique of quantifying erythema by measuring the amount of green light re-emitted from the skin *in vivo* is not new. Tronnier (1969) reviewed various instruments that were designed and used for this purpose, but due to limitations in technology, these instruments were probably too cumbersome and imprecise for routine clinical use. Nevertheless, simple photoelectric reflectance meters which evolved from these early instruments enabled useful data to be obtained regarding the quantitation of erythemal responses to ultraviolet radiation (Breit & Kligman, 1969; Daniels & Imbrie, 1958).

With the advent of computer-controlled spectrophotometers, reliable measurements of the spectral reflectance of human skin *in vivo* have been achieved (Dawson *et al.*, 1980; Wan, Jaenicke & Parrish, 1983a; Wan, Parrish & Jaenicke, 1983b). Although this type of instrumentation can provide full diffuse reflectance spectra over the ultraviolet and visible regions, the time required for scanning and recording at each site, and the bulkiness and expense of the equipment present limitations to the acceptability of the technique.

Since there does not appear to be a satisfactory commercially available instrument for

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measuring the degree of cutaneous erythema, we decided to design and construct a device incorporating modern electro-optical technology. A prerequisite of the device was that it must be able to record quickly, easily and precisely the degree of erythema at any anatomical site. We were prepared to sacrifice high specificity, and a single measurement of the skin with our instrument does not fully discriminate between erythemal and pigmentary changes, although the ratio technique which we adopted partly compensates for this. Nevertheless, if the instrument is used to record an 'erythema index' at a site on the skin before and after irradiation it is shown that the difference between those two readings is essentially related to an increase in vasodilation and is independent of the melanin content of the epidermis.

THE INSTRUMENT

Principle of operation of the instrument

The major chromophores for visible light in the epidermis and dermis are melanin and haemoglobin, respectively. Oxyhaemoglobin shows high absorption of light in the spectral range 520–580 nm (green light). With increasing wavelength the absorbance falls rapidly so that absorption in the red part of the visible spectrum is minimal (Fig. 1). When the blood content of the subpapillary plexus increases, resulting in erythema, a greater amount of green light is absorbed and less is reflected. In contrast, the amount of red light absorbed or reflected shows little change with vasodilation.

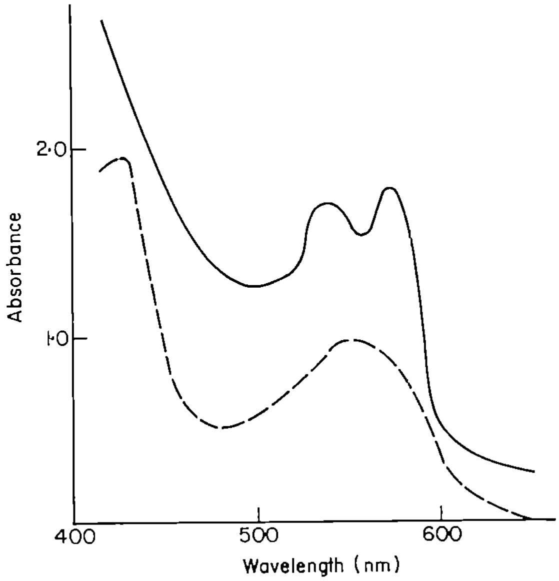

FIGURE 1. The absorption spectra of oxygenated (——) and reduced (– – –) blood solution.

The following analysis, on which the principle of operation of this instrument is based, shows that by comparing the amount of reflected red and green light, an index may be obtained which depends mainly on the blood content of the superficial dermis. This 'erythema index' may be defined as follows.

$$\text{Erythema index} = \log_{10}\left(\frac{\text{Intensity of red component of reflected light}}{\text{Intensity of green component of reflected light}}\right) \qquad (1)$$

The normal skin is illustrated schematically in Fig. 2. It may be shown (Dawson *et al.*, 1980; Anderson, Hu & Parrish, 1981) that the reflectance (R_g) of green light (530–560 nm) from skin *in vivo* can be approximated as

$$R_g \simeq r + (1-r)\,(R_e + T_e^2 R_d + T_e^2 T_d^2 R_s) \qquad (2)$$

where r is the specular, or regular, reflectance from the surface of the skin and is due to the difference between the refractive index of air (1·0) and stratum corneum (1·55); R_e and T_e are the diffuse reflectance and transmittance of the epidermis, respectively; R_d and T_d are the diffuse reflectance and transmittance of the layer of the dermis containing the subpapillary vascular plexus, respectively; and R_s is the diffuse reflectance of the dermal layer and subcutaneous fat beneath the superficial plexus.

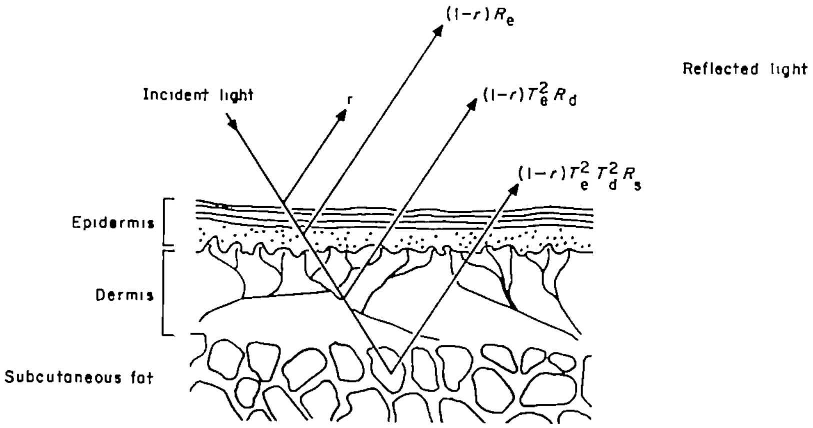

FIGURE 2. A schematic diagram of human skin showing the reflection and transmission of light from each layer.

For photons with wavelengths throughout the visible spectrum (400–700 nm) and for both fair and dark skin (Wan *et al.*, 1983b), $r \ll 1$, $R_e \ll 1$ and $R_d \ll 1$.

If we assume, therefore, that r, R_e and R_d all approach zero, then equation (2) can be simplified as

$$R_g \simeq T_e^2 T_d^2 R_s \qquad (3)$$

Since the transmittance of haemoglobin bears an exponential relationship to its extinction coefficient (Anderson & Sekelj, 1967) we can write

$$T_d = 10^{-A/2} \qquad (4)$$

where A is a parameter relating to the absorbance of the blood in the subpapillary vascular plexus. By taking the logarithm to the base 10 of the inverse reflectance given by equation (3) and substituting equation (4) into it we obtain:

$$\log(1/R_g) = -\log(T_e^2) + A - \log(R_s). \qquad (5)$$

If the reflectance (R_r) of red light (660–690 nm) is measured simultaneously we obtain an equation analogous to equation (5) where the terms T_e, A and R_s now, of course, refer to red light.

We may note further that:

(1) the extinction coefficient of haemoglobin for green light is considerably greater than the coefficient for red light. Hence $A_g - A_r \simeq A_g$, where the suffixes g and r refer to green and red light, respectively.

(2) The transmittance (T_e) of green light through fair-skinned Caucasian epidermis is approximately 0·75, rising to about 0·80 for red light (Wan *et al.*, 1983b).

(3) The reflectance (R_s) of green light from the tissue beneath the superficial plexus is approximately 0·60, rising to about 0·75 for red light (Wan *et al.*, 1983b).

Consequently, by subtracting the logarithm of the inverse reflectance of red light from that of green light we can obtain an erythema index which minimizes the influence of epidermal melanin and reflection of light from the tissue layer beneath the superficial plexus, and is expressed as

$$\begin{aligned}\text{Erythema index} &= \log(^1/R_g) - \log(^1/R_r)\\ &= \log\,(R_r/R_g). \qquad (6)\end{aligned}$$

This expression is, of course, identical to equation (1). A further benefit of the ratio method is that it eliminates the effect of variation in distance between the end of the fibre optic cable used to conduct light to and from the skin and the surface in question; this independence would clearly not apply if only the reflectance of green light was measured.

Finally, if the erythema index determined on each skin site prior to irradiation is subtracted from the erythema index determined on each corresponding site after irradiation, and if it is assumed that T_e and R_s for both red and green light remain unchanged (which is probably true for up to 2 days after irradiation if immediate pigment darkening is ignored), we obtain a difference which is directly related to the absorbance of blood in the subpapillary vascular plexus, that is, the degree of cutaneous erythema, and independent of the optics of the epidermis and the tissue layer beneath the superficial plexus.

General description

The white light from a projector bulb is focussed on to the aperture of one branch of a trifurcated fibre optic cable. The common leg of the fibre optic cable is supported 1 mm from the skin surface in a conically-shaped applicator of 21 mm diameter (Fig. 3). This is appreciably greater than the diameter (5·5 mm) of the light guide and, consequently, the reading of the instrument is unaffected by changes in applied pressure within reasonable operational limits. Light re-emitted from the skin passes up the other two branches; one of these is optically coupled to a green interference filter and photodiode, and the other is optically coupled to a red interference filter and photodiode. The photocurrents from both photodiodes are amplified and form the two inputs to a log ratio module. The output from this module is displayed as the erythema index.

An external zero control adjusts the gain of one of the amplifiers such that when the applicator attached to the common leg of the fibre optic cable is in contact with a white diffuse reflectance plaque [Eastman white reflectance paint ($BaSO_4$)], both inputs to the log ratio module are made equal resulting in a displayed erythema index of zero. The instrument may be used in normal room lighting. A full technical description is given in the appendix.

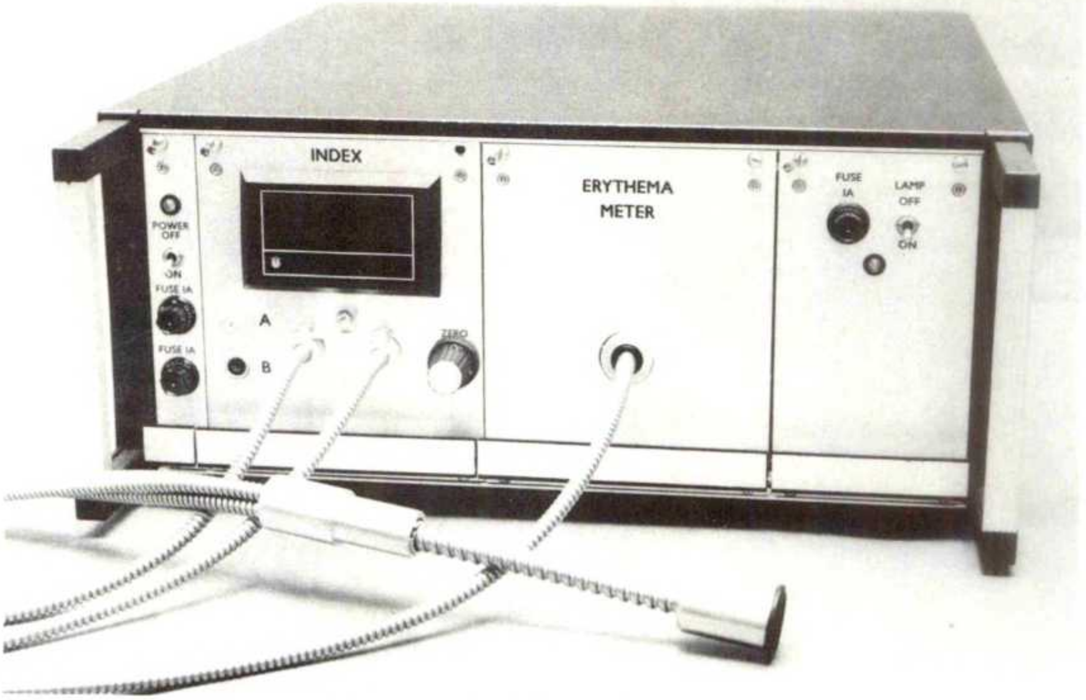

FIGURE 3. A photograph of the instrument for quantifying erythema.

Stability of the instrument

The fibre optic cable was placed in contact with the white diffuse reflectance plaque and the erythema index monitored using a chart recorder over a period of several hours. The erythema index drifted by 0·003 units during the first hour and thereafter by less than 0·001 units per hour. The same results were obtained when the fibre optic cable was placed in contact with a matt pink card with an erythema index of 0·530.

Comparison of the threshold sensitivities of the instrument with the eye

Samples of white filter paper were immersed in different known concentrations of red ink diluted with water and allowed to dry. Four independent observers were asked to judge which sample of filter paper appeared to be a just discernible hue viewed against a white background. The erythema index of each sample was then measured with the instrument. It was found that the instrument recorded a positive erythema index at dilutions approximately one order of magnitude higher than were detected by eye (Fig. 4). This finding is borne out in the *in vivo* studies described by Farr & Diffey (1984) in which the instrument detects erythema at ultraviolet radiation doses below the minimal erythema dose.

Validation of the instrument

The instrument is quick and simple to use *in vivo*, each measurement taking only a few seconds to perform. In normal use, a coefficient of variation of about 3% may be obtained by repeated measurements at a single site.

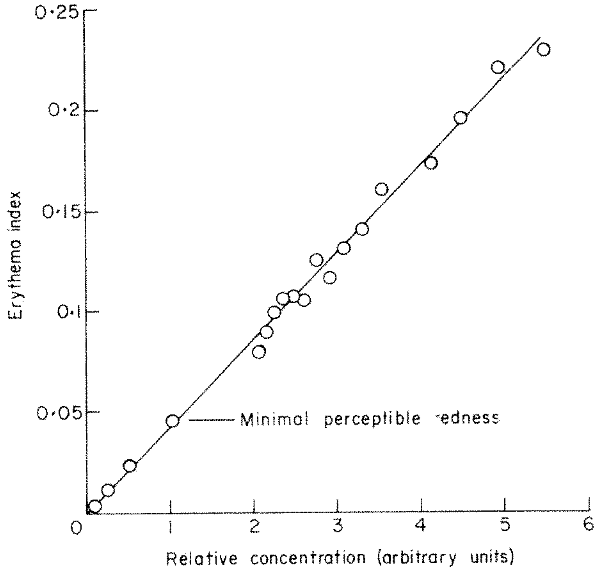

FIGURE 4. The erythema index as a function of red ink concentration in water.

Currents generated by the two photodiodes with the instrument in use, and the corresponding calculated indices of erythema are shown in Table 1. The current from the red detector is only slightly higher in the untanned subject and in both subjects it remains virtually constant, irrespective of change in erythema. The current generated by the green detector is likewise higher in the untanned subject, but in both subjects it varies inversely with erythema. These observations are in keeping with the conclusions of the mathematical analysis developed earlier.

It is difficult to use the skin *in vivo* to validate further the performance of the instrument, since the degree of vasodilation in the subpapillary vascular plexus and the content of melanin

TABLE 1. Typical photocurrents and erythema indices from different surfaces

Surface	Photocurrent, μA		Erythema index
	Red detector	Green detector	
Matt black felt	0·03	0·03	—
White diffuse reflectance standard	1·04	1·06	0·000
Untanned Caucasian skin			
No erythema	0·51	0·40	0·114
Moderate erythema	0·50	0·26	0·292
Marked erythema	0·49	0·22	0·356
Tanned Caucasian skin			
No erythema	0·45	0·30	0·184
Moderate erythema	0·44	0·23	0·290
Marked erythema	0·43	0·18	0·386

pigment in the epidermis are both unknown. Consequently, a simple model was constructed as described below.

A set of equally spaced parallel red lines were drawn in a 4-cm square grid on a sheet of white paper using a computer-controlled pen to ensure high accuracy. The separation between each line was 2·0 mm. Eight further sets were drawn with line separations of 1·8, 1·6, 1·4, 1·2, 1·0, 0·8, 0·7 and 0·6 mm. The reciprocal of the line separation is directly related to the red content per unit area or 'degree of erythema'. In order to simulate varying degrees of pigmentation ranging from amelanotic to Negro skin, a clear plastic sheet, a light bronze-coloured plastic sheet and a dark bronze-coloured plastic sheet were placed over each grid in turn. The transmittance of red light (672 nm) and green light (546 nm) through the light bronze-coloured sheet was 0·65 and 0·61, respectively, and approximates the transmittance of red and green light through lightly tanned Caucasian epidermis (Wan *et al.*, 1983b). The transmittance of red and green light through the dark bronze-coloured sheet was 0·35 and 0·17, respectively, and approximates to the transmittance of Negroid epidermis (Wan, Anderson & Parrish, 1981). For each plastic sheet, the photocurrent generated in the red detector hardly changed with the different grids, unlike the photocurrent from the green detector which decreased with decreasing separation of the red lines.

The measured 'erythema indices' are plotted against the reciprocal of the red line separation in Fig. 5. The range of erythema indices is equivalent to that found in unexposed to severely

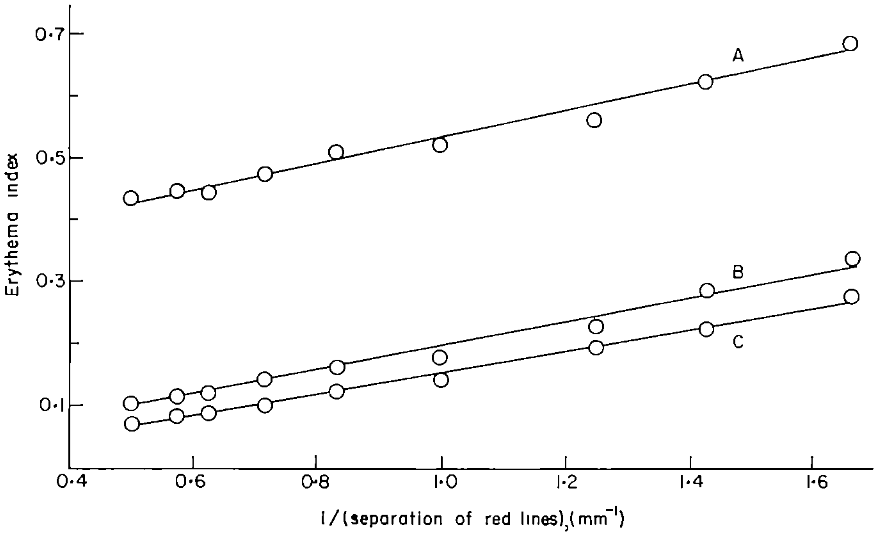

FIGURE 5. The measured erythema indices as a function of the reciprocal of the red line separation for three different coloured plastic sheets overlying the red grids.

Curve	Colour of plastic sheet	Pigmentation equivalent	Slope	Correlation coefficient
A	Dark bronze	Black skin	0·207	0 987
B	Light bronze	Tanned skin	0·190	0·992
C	Clear	Amelanotic skin	0 170	0 996

erythematous skin *in vivo*. It may be seen that the absolute value of the erythema index at a given line separation (equivalent to a hypothetical degree of vasodilation) increases with degree of pigmentation, as predicted from equation (5). However, the slopes of each of the three curves are linear and approximately equal, implying that the difference between the post- and pre-irradiation erythema indices at a given skin site is directly proportional to the amount of cutaneous blood per unit area and independent of the melanin content of the epidermis.

This portable instrument will quickly and easily quantify cutaneous erythema. Its performance in quantifying UVR-induced erythema in skin *in vivo* is described by Farr & Diffey (1984).

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APPENDIX

Technical description of the instrument

A1. Optical components

A.1.1. Lamp and supply

The lamp used is a 75 W quartz tungsten halogen projector bulb (Wotan, type Xenophot HLX) which operates at a constant colour temperature and constant luminous flux during the rated lamp life of 50 h. The lamp is mounted at the focus of an ellipsoidal dichroic mirror and the entrance aperture of the fibre optic cable positioned close to the virtual focus of the mirror. A heat-absorbing glass filter is placed between the lamp and fibre optic cable.

In order to ensure a stable operating temperature, which implies a constant spectral emission, it was necessary to operate the lamp from a simple 12 V stabilized power supply utilizing an LM78H12K regulator to eliminate supply instabilities associated with the mains supply fluctuations.

A.1.2. Fibre optic cable

A trifurcated glass fibre optic cable (Oriel Corporation, Stamford, Conn.) is used to conduct the light to and from the skin. The cable is 916 mm in length; the diameter of the fibre bundle of each branch is 3·2 mm, with a common leg bundle diameter of 5·5 mm. The fibre optic cable has an acceptance angle of 68° and a numerical aperture of 0·55. The individual fibres of the three branches are randomly mixed at the skin surface.

A.1.3. Interference filters

The two interference filters used (type VIS-14: Barr & Stroud Ltd., Glasgow) had peak transmissions at 546 nm (green light) and 672 nm (red light), respectively, and both had a band width at half peak of 14 nm. These wavelengths were chosen since there is a local peak in the absorption spectra of both reduced haemoglobin and oxyhaemoglobin at wavelengths around 546 nm, with negligible absorption at wavelengths greater than about 650 nm.

A.1.4. Photodiodes

The detector for the green light was a Hammamatsu gallium arsenide phosphate (GaAsP) Schottky photodiode (type no. G1126) with a peak sensitivity at 580 ± 30 nm falling to a sensitivity of 5% of the peak at 180 nm and 680 nm. The advantage of this photodiode is that it has no infra-red response and so will have a negligible response to photons with wavelengths in the red and near infra-red regions transmitted as background radiation through the green interference filter.

A silicon photodiode (type no. S874-5K: Hammamatsu) was used to detect the red light. This photodiode has a peak wavelength sensitivity at 850 nm with a relative sensitivity of 80% of the peak at a wavelength of 672 nm (the peak wavelength of the red interference filter).

A.2. Analogue electronics

A circuit diagram of the electronic components used in the instrument is shown in Fig. 6. The electrical signals from each photodiode were fed into each half of a CA3240 BiMos operational amplifier which is operated in the inverting mode in order to monitor directly the photocurrents generated by each photodiode. This device was chosen as it meets the specifications of high input–impedance (1·5 TΩ) and low input bias currents (20 pA) required by the low photocurrents generated in the photodiodes (see Table 1).

It should, however, be noted that in order to obtain a linear current/radiation–intensity relationship the photodiode must be operated in the photoconductive mode under zero- or reverse-bias conditions. In this application, the zero-bias condition has been selected for simplicity. It is, therefore, necessary to ensure that all voltage offsets appearing at the noninverting input are kept as small as possible. If this is not done then there is the possibility of biasing the diode into the logarithmic photovoltaic region. Thus, the offset null facility is employed to ensure that the device offset voltage (typically 5 mV) is reduced to less than 1 mV. The input offset voltage drift with temperature of 8 μV/°C ensures that any temperature effects are negligible.

The output from the detector amplifier is passed into a passive 50 Hz notch filter which reduces the mains signal picked up by the high-impedance photodiodes. An impedance-buffer amplifier is interposed between the filter and the following sections to prevent loading of the output of the filter.

The output of the green signal from the buffer amplifier is fed through a unity gain amplifier with adjustable offset into the reference input channel of a six-decade, high accuracy logarithmic ratio module (type 757N: Analog Devices). The red signal passes through an amplifier with an adjustable gain control into the signal input channel of the log ratio module. The gain control is used to equalise the input signals to the log ratio module when the fibre optic cable is placed in contact with the white diffuse reflectance plaque. This results in a display of 0·000 on the digital voltmeter used to indicate the erythema index.

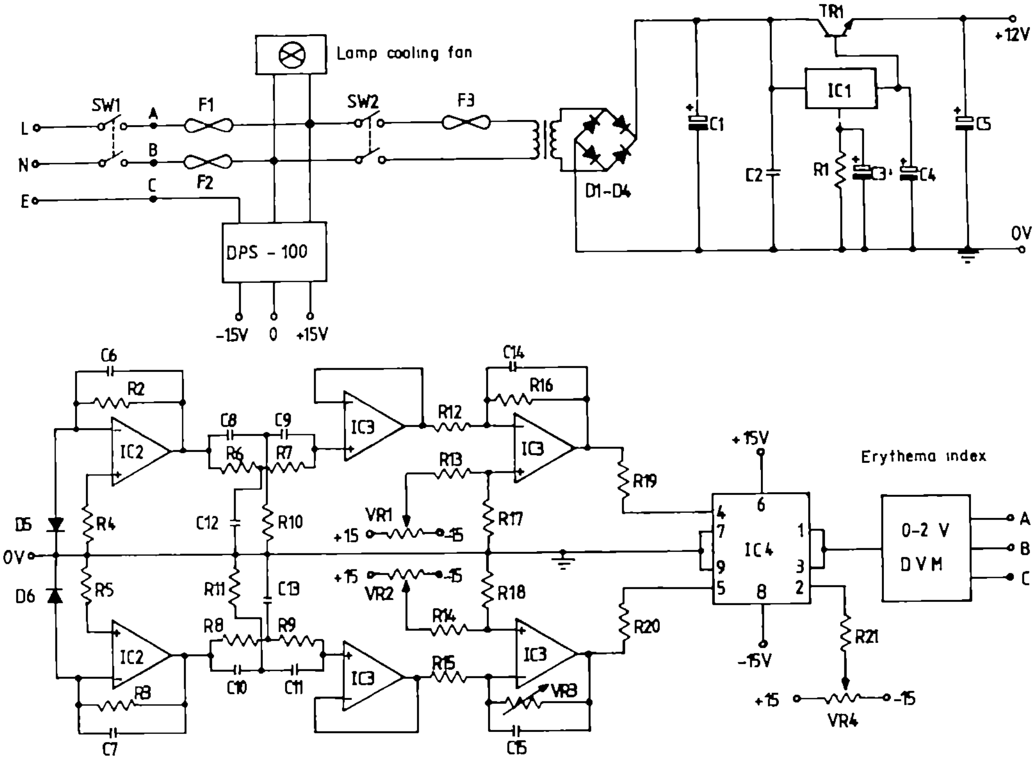

FIGURE 6. The circuit diagram of the electronic components used in the instrument.

Component list

Resistors	
R1	68 Ω
R2	1 MΩ
R3	1 MΩ
R4	1 MΩ
R5	1 MΩ
R6	33 kΩ
R7	33 kΩ
R8	33 kΩ
R9	33 kΩ
R10	27 kΩ
R11	27 kΩ
R12	1 kΩ
R13	150 kΩ
R14	150 kΩ
R15	1 kΩ
R16	1 kΩ
R17	1 kΩ
R18	1 kΩ
R19	1 MΩ
R20	1 MΩ
R21	200 kΩ

Variable resistors	
VR1	10 kΩ
VR2	10 kΩ
VR3	1 kΩ
VR4	20 kΩ

Capacitors	
C1	10,000 μF
C2	0·1 μF
C3	10 μF
C4	10 μF
C5	20 μF
C6	300 pF
C7	300 pF
C8	0·1 μF
C9	0·1 μF
C10	0·1 μF
C11	0·1 μF
C12	0·22 μF
C13	0·22 μF
C14	0·1 μF
C15	0·1 μF

Semi-conductors	
TR1	2N3055
IC1	78H12K
IC2	CA3240
IC3	LM324
IC4	757N

D1–D4 1A Bridge Rectifier

D5 GaAsP photodiode

D6 Si photodiode

Fuses	
F1	1A
F2	1A

Switches	
SW1	3A
SW2	3A

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Quantitative studies on cutaneous erythema induced by ultraviolet radiation

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SUMMARY

A reflectance instrument was used to measure the variation in UVR-induced erythema at different positions on the back. The pre-irradiation erythema index decreased from top to bottom of the back but the increase in index remained constant for a fixed exposure dose. In contrast, the minimal erythema dose was higher at lower sites on the back. The measured erythemal response increased linearly with the logarithm of the radiation dose from approximately the minimal erythema dose up to at least fifteen times this value.

The erythemal response of the skin to ultraviolet radiation (UVR) is usually inferred from the minimal erythema dose (MED). The visual detection of erythema is subjective and is affected by several unrelated factors such as viewing geometry, ambient illumination, colour of unexposed surrounding skin (Chamberlin & Chamberlin, 1980) and also the experience and visual acuity of the observer. The difficulty in accurately judging a minimal erythema response is reflected by the varying definitions proposed for this value which range from the dose required to initiate a faint but easily discernible erythema (Epstein, 1962) to that dose which will just produce a uniform redness with sharp borders (Willis & Kligman, 1970). More importantly, the MED is an apparent threshold measurement and may tell little about the erythemal response to other doses. An instrumental method of accurately quantifying erythema would allow the construction of a dose-response curve and also eliminate the problem of subjective measurement.

This paper describes the objective measurement of the cutaneous erythemal response to UVR and compares this with information obtained from conventional, subjective and visual estimation of the MED.

METHODS

Patients

Adult Caucasian volunteers of different skin types were studied; all gave informed consent. None had any history to suggest abnormal sensitivity to UVR and none were taking any drugs

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known to provoke such a reaction. Only one subject (PB) showed an obvious variation in pigmentation on the back, with a gradual reduction in tan from top to bottom.

Photoirradiation apparatus and radiation dosimetry

The optical radiation source used was an unfiltered xenon arc lamp (900 W) mounted in a housing which formed part of a clinical photoirradiation apparatus (Diffey, Farr & Ive, 1984). A liquid-filled light guide, 2 m in length and 5 mm inner diameter, conducted the radiation from the exit port of the lamp housing to the patient's skin. An applicator which housed two fused silica lenses was attached to the proximal end of the light guide and produced a spatially uniform beam of radiation, 15 mm in diameter, on the patient's skin.

The spectral power distribution of the radiation incident on the patient's skin was measured using a scanning spectroradiometer (Fig. 1). The irradiance was measured using a solar-blind vacuum phototube and multilayer interference filter combination which had a spectral response closely approximating the erythema action spectrum for human skin. This detector was calibrated at a wavelength of 300 nm and so the erythemally effective UVR doses [termed UVR(EE)] quoted here can be thought of as equivalent to that hypothetical dose of 300 nm radiation which would produce the same degree of erythema as the exposure to the xenon lamp.

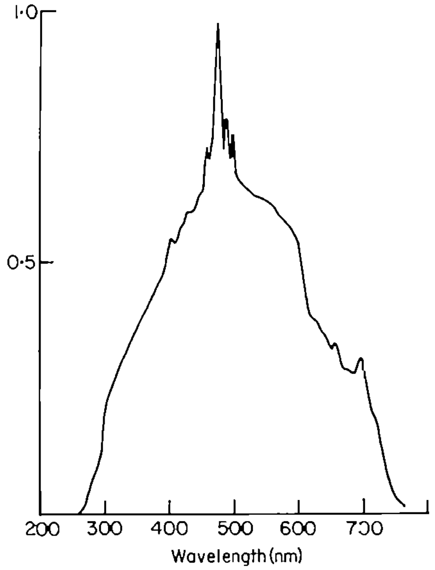


FIGURE 1. The spectral power distribution (relative units) of optical radiation incident on the subjects' skin.

Erythema measurement

Erythema was measured with a reflectance instrument (Diffey, Oliver & Farr, 1984) which obtains an index related to blood content of the superficial dermis. This 'erythema index' is defined as

$$\log_{10}\left[\frac{\text{Intensity of red component of reflected light}}{\text{Intensity of green component of reflected light}}\right].$$

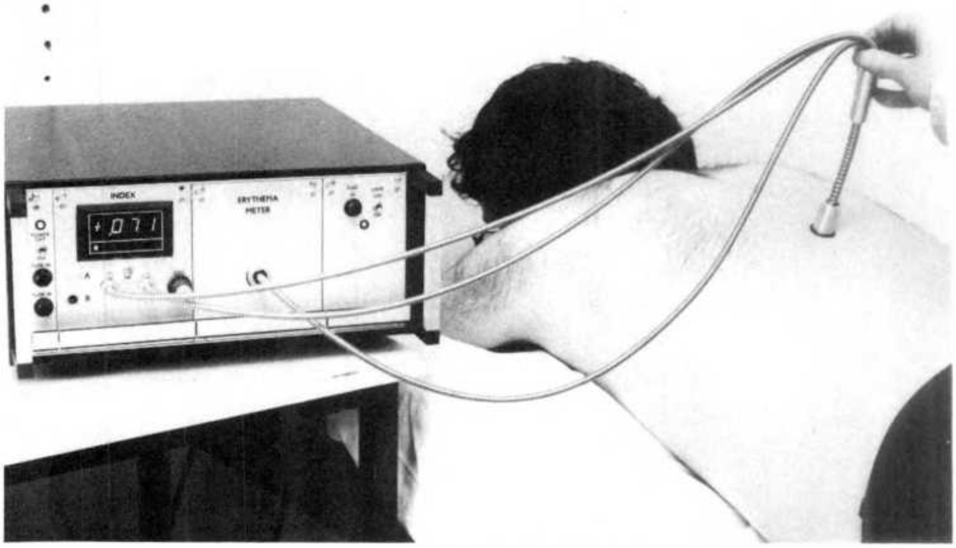


FIGURE 2. The measurement of erythema on the back of a subject using the instrument.

Three measurements were taken at each site under study with the subject lying prone on a couch (Fig. 2).

Variation in erythemal response with anatomical site

Twelve sites, six on each side of the body (Fig. 3), were studied in five subjects (three female, two male, age range 20–44 years). All exposures were made on sites located between 4 and 7 cm from the midline of the back, on either side of the body. The vertical distance between sites was approximately equal, and was related to vertebral level. The erythema index was measured at each marked site. The sites were then consecutively exposed to radiation, the UVR(EE) dose being constant for each subject but varying from 104 mJ/cm^2 to 168 mJ/cm^2 between subjects, depending on their sun-reactive skin type. The measurements of erythema index were repeated 24 h later.

Variation in MED with anatomical site

The MED was determined in five subjects (one female, four males, age range 20–35 years) at three sites on the back related to vertebral level; upper dorsal (T3), lower dorsal (T9) and lumbar (L2) regions. Exposure time increments of 1 sec were used and the shortest exposure time noted that produced a just perceptible erythema, 24 h after irradiation.

Dose-response studies

Two male subjects aged 26 and 34 years were studied. Eleven sites across the back at the level of T8–T10 were exposed to increasing UVR(EE) doses varying from zero to 720 mJ/cm^2. The pre- and post-irradiation erythema indices were measured as described previously and, in addition, the approximate 24-h MED was assessed visually.

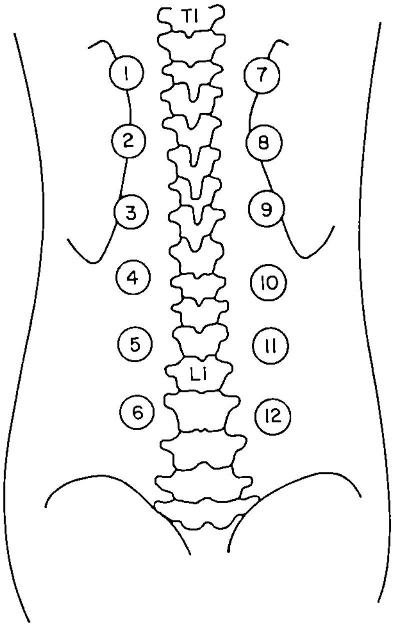


FIGURE 3. A plan of the back showing the twelve irradiation sites in relation to the vertebrae.

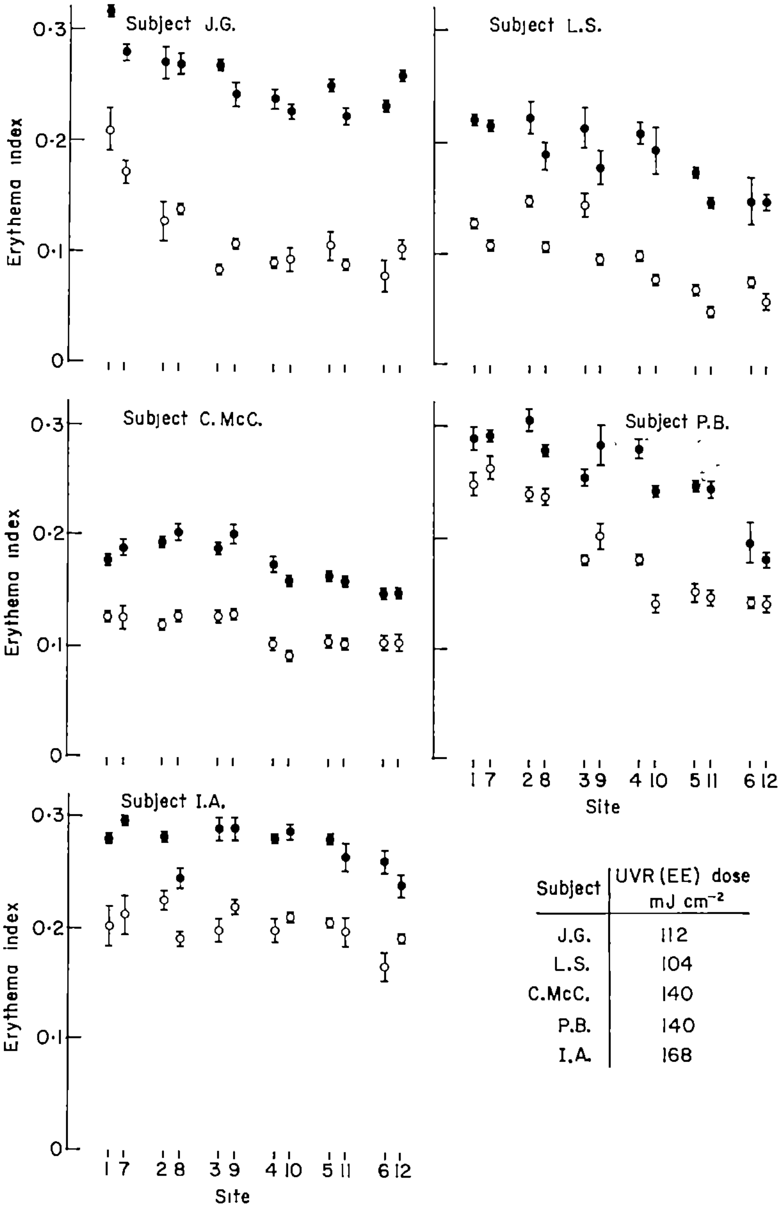
RESULTS

Variation in erythemal response with anatomical site

The results obtained for each subject are shown in Fig. 4. The pre-irradiation erythema index in all cases showed a considerable variation with site despite there being no visible difference in erythema. In every case there was a gradual reduction in pre-irradiation erythema index from upper to lower back, although the degree of reduction varied from subject to subject. The correlation between left and right sides of the back was generally good with an average coefficient of variation of 6%, compared with 22% measured in the vertical plane.

The degree of erythema judged by eye 24 h after irradiation also showed marked variation even though the same dose of UVR had been used at each site. In all cases it was noticeably more intense at the upper back compared with lower sites where, in some subjects, the erythema was barely detectable. The post-irradiation indices also demonstrated a gradual reduction from upper to lower sites.

Table 1 summarizes the erythema indices obtained on each subject and also examines the difference between post- and pre-irradiation measurements. Standard statistical formulae were used to calculate the intra-site, inter-site and overall standard errors (Campion, Burns & Williams, 1973). It will be seen that in each case the standard error based on the variation within each irradiation site is considerably lower than the standard error based on the variation between each irradiated site. The total standard error of the mean value averaged over all sites is always lower if the difference between post- and pre-irradiation indices is used, rather than either set of indices alone. The subject (PB) who had an obvious variation in melanin pigmentation on the back had the highest overall standard error.



Subject	UVR (EE) dose mJ cm^{-2}
J.G.	112
L.S.	104
C.McC.	140
P.B.	140
I.A.	168

FIGURE 4. The mean erythema indices measured on each of the twelve sites for each of the five subjects. ○: pre-irradiation values; ●: post-irradiation values; the error bars represent one standard deviation about the mean.

Variation in MED with anatomical site

The results obtained are shown in Table 2. In every subject the exposure time for a minimal erythema at the lower back was considerably higher than at the other sites. In every subject the MED was increased by almost twofold from top to bottom of the back.

TABLE 1. Summary of erythema indices obtained on each subject: pre-irradiation, post-irradiation and the difference

				Erythema index (× 1000)											
				Pre-irradiation				Post-irradiation				Difference			
Subject	Skin type	Degree of pigmentation*	UVR(EE) mJ/cm^2	μ	σ_1	σ_2	σ	μ	σ_1	σ_2	σ	μ	σ_1	σ_2	σ
JG	I	0	112	117	1·8	11·2	11·4	256	1·3	7·6	7·8	139	2·2	6·2	6·6
LS	II	+	104	96	1·0	9·1	9·2	186	2·6	8·6	9·0	90	2·8	4·4	5·2
CMcC	II	+ +	140	113	0·8	3·9	4·0	174	0·9	5·6	5·7	62	1·2	3·1	3·3
PB	III	+ +	140	188	1·4	13·6	13·6	256	1·6	11·4	11·5	68	2·2	8·0	8·3
IA	IV	+ + +	168	202	1·8	4·4	4·8	275	1·4	5·2	5·4	73	2·2	4·3	4·8

μ: Mean value average over twelve irradiation sites on back.

σ_1: Standard error of μ based on variation within each irradiation site.

σ_2: Standard error of μ based on variation between each irradiation site.

σ: Total standard error of μ ($\sigma^2 = \sigma_1^2 + \sigma_2^2$).

* Key: 0, no discernible tan; +, barely perceptible tan, + +, light tan; + + +, medium tan.

TABLE 2. Exposure times (sec) for a minimal perceptible erythema (MPE) at 24 h after irradiation on different regions of the back

	Time for MPE (s)		
Subject	Upper dorsal	Lower dorsal	Lumbar
PF	5	4	9
BD	7	9	12
DS	8	5	11
CG	6	6	10
CMcC	4	4	7

Dose-response studies

UVR dose–erythemal response curves were obtained in two subjects (Fig. 5). For each subject two curves are plotted; one based on post-irradiation erythema indices alone, and the other using the difference between post- and pre-irradiation measurements. The initial part of the dose-response curve is non-linear. From the approximate region of the MED a linear response is seen. Flattening of the upper part of the curve may have occurred in one subject (SZ), although this is less obvious on the graph when the differences between post- and pre-irradiation indices are plotted. In both subjects the degree of erythema at the three highest doses (360–720 mJ/cm^2) appeared equally red to the eye, despite the erythema index continuing to rise. The correlation coefficient determined using the linear part of the curve is always higher if the difference between indices is used rather than the post-irradiation values alone.

DISCUSSION

The standard errors of both the mean pre-irradiation erythema index and the mean post-irradiation index are very small. Typically, the coefficient of variation of a single reading on the skin is 3%. This is larger than the coefficient of variation obtained on an inert surface such as matt pink card (less than 1%) and is due to small changes in re-positioning the fibre optic cable at each of the marked sites on the skin. A coefficient of variation of 3% is considerably smaller than the dosage increments of 20% or more generally used in the determination of the MED. In fact, some workers (Kimmig & Wiskemann, 1959) have suggested that a reliable estimate of a normal sunburn MED determination cannot be achieved to much better than ±20%. This factor implies that a quantitative measurement of erythema will be a more accurate indicator of vasodilation than the MED.

The appreciable decrease in pre-irradiation erythema index from top to bottom of the back is thought to reflect regional variation in blood vessel architecture and blood flow. Although degree of erythema is a poor guide to total cutaneous blood flow, skin colour is influenced by blood flow at the dermal-epidermal junction (Millington & Wilkinson, 1983). In keeping with our findings there is a small but significant decrease in blood flow as measured by laser Doppler velocimetry between sites on the upper and lower trunk (Tur *et al.*, 1983).

The observation that in every subject the difference between the post- and pre-irradiation erythema indices at each site showed much less variation than either set of indices considered alone suggests that anatomical variation of melanin concentration and epidermal thickness over the back must have been very small. If this were not the case, the optical filtering properties of either of these two factors would have resulted in significant differences in the degree of vasodilation produced at each site. In accordance with this, the only subject who had an obvious variation in melanin pigmentation on the back also had the highest overall standard error.

The MED showed considerable variation over the back. In each case the value obtained was considerably higher at lower sites than for other areas. The erythemal response of the skin to UVR is known to depend on anatomical position. Olson, Sayre & Everett (1966) found the 8-h MED to vary by a factor of four depending on body site. The trunk, head and neck were more sensitive than the extremities and it was concluded that the trunk is best suited for study because of higher sensitivity and more uniform response. Little data is available, however, concerning the MED with regard to precise anatomical location on the back. Our results are at variance with those of Tronnier (1969) who found caudal sites more sensitive than cranial sites. No further anatomical details are given but the discrepancy could be explained if the caudal sites were normally sun-protected buttock skin. Other studies have tended to support our findings. Barth & Jacobi (1979) found the MED to be lower in the scapular region than at lumbar sites, but did not provide any quantitative data. Frain-Bell & Johnson (personal communication, 1983) have observed the same effect using monochromatic radiation at 300 and 305 nm, although the overall variation was considerably less than in our subjects.

Our results suggest that if the MED is to be used as an index of skin reactivity to UVR, the irradiated sites should be as closely apposed as possible in order to avoid appreciable anatomical variation. In addition, details of the exact site of testing should be recorded to enable valid comparisons to be made either between subjects or between serial results obtained on a single subject.

The back has a relatively uniform response to UVR providing the difference between post- and pre-irradiation indices is recorded. It is surprising, therefore, that the MED is considerably higher at lower sites on the back. This would suggest that the ability of the eye to detect a just noticeable difference in erythema varies according to site on the back. One factor involved may be the change in background erythema over this area. Background erythema can be shown to alter the sensitivity of the eye by observation of inner forearm skin exposed to a series of increasing doses of UVR. The MED is first assessed with the arm in horizontal position. If the arm is then elevated, the background skin erythema index falls, but the differences in erythema indices between exposed sites and background skin remain constant. The MED determined with the arm elevated is higher than that previously obtained with the arm horizontal.

A value, such as the MED, is merely one point on a dose-response curve. It can be expected, therefore, that the full curve will yield very much more information than does a single point. Magnus (1976) demonstrated a linear response by re-plotting on a log–dose scale the results obtained by other workers on erythema quantification using red photographic filters. Our data support this finding; in both subjects the middle part of the curve is linear to a high degree (both correlation coefficients equal to 0·99). Even though UVR(EE) doses of up to fifteen times the MED were given, resulting in painful burns, no clear flattening off of the curve was seen. The time after irradiation of appearance and disappearance of erythema is known to be related to exposure dose of UVR (van der Leun, 1966). It is to be anticipated, therefore, that the form of the dose-response curve will depend upon the time of measurement. From about 8 to 48 h after irradiation we have found that the change in gradient of the erythemal response–log dose curve is minimal.

It is noteworthy that the MED lies at the lower end of the linear part of the curve and is therefore a detection rather than a response threshold. The increase in erythema index that occurs with lower doses of UVR than the visual MED is attributed to small changes in blood content that are not apparent to the eye. This agrees with the results on an artificial model system which indicated that the instrument is more sensitive than the eye at detecting low concentrations of red pigment (Diffey *et al.*, 1984).

Dose-response curves of ultraviolet erythema have recently been published by Wan, Parrish & Jaenicke (1983) and Wan, Jaenicke & Parrish (1983) who used a scanning reflectance spectrophotometer. These authors did not determine the degree of basal erythema at each site, an omission which our data suggest could lead to appreciable perturbations in the dose-response curve. Also, their dose-response curves, plotted on a linear dose scale, indicate that the degree of both UV-B and UV-A erythemas measured at 24 h after irradiation reach a plateau at about three times the MED (defined as erythema with definite borders).

Objective measurements and dose-response data obtained by this reflectance instrument are likely to yield more information than the MED concerning the erythemal response of individuals with normal skin and those with the various photodermatoses. More precise evaluation of photoprotective agents and treatment regimens should also be possible.

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Case Reports

Neonatal lupus erythematosus in four successive siblings born to a mother with discoid lupus erythematosus

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SUMMARY

A young woman with inactive discoid lupus erythematosus (LE) gave birth in three successive pregnancies to four male infants who showed cutaneous, and in one case cardiac, signs of neonatal LE. The mother had Ro and La antibodies although the anti-nuclear factor (ANF) was not consistently detectable. Three of the infants received phototherapy for neonatal jaundice. Maternal discoid LE may give rise to neonatal LE, and successive siblings can be affected.

Neonatal LE is now a well-recognized syndrome which may involve the cutaneous, cardiac or haematological systems, either singly or in a multiorgan fashion. Many of the mothers have an underlying connective tissue disorder, usually systemic LE or Sjögren's syndrome (McCuistion & Schoch, 1954; Vonderheid *et al.*, 1976; Esscher & Scott, 1979), but some are asymptomatic (Weston *et al.*, 1982). Recent studies have demonstrated that the mothers, including those who are asymptomatic, have serum antibodies to Ro, La or both Ro and La antigens (Weston *et al.*, 1982). These antibodies have been found in the serum of affected infants, suggesting placental transmission of maternal antibodies (Franco *et al.*, 1981). Such women are at risk of producing further babies with neonatal LE, and we report the successive occurrence of this syndrome in four siblings, who are remarkable because they are all male.

CASE REPORTS

Case 1

A 26-year-old female developed a facial 'butterfly' rash in 1975 and was diagnosed as having discoid LE 5 months later. Her ANF test was positive at 1/100 but the DNA antibody test was normal at 4%. Her rash cleared after a 6-week course of chloroquine and she has had no further

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cutaneous or systemic symptoms. ANF tests repeated in 1977, 1979 and 1982 were negative, but antibodies to Ro and La were demonstrated in 1984; RNP and Sm antibodies were not found. The patient has had three full term normal pregnancies, being delivered of male twins in 1977 (cases 2 and 3), and male infants in 1979 and 1981 (cases 4 and 5). All births were by caesarean section, the first having been performed because of fetal bradycardia.

Case 2

This male infant presented at 8 weeks of age with a Stokes Adams attack and second degree heart block. He had a rash on his right temple which subsequently faded after the age of 15 weeks. A full blood count, carried out when he was 2 months old, showed a haemoglobin of 8·8 g/dl with fragmented red cells and schistocytes on the blood film. The white cell count and platelet count were normal. Both he and his brother (case 3) received phototherapy with light of wavelength 400–475 nm, for a few days after birth because of neonatal jaundice. After several Stokes Adams attacks an epicardial demand pacemaker was inserted when he was 11 months old. An ANF test performed at the age of 2 years 9 months was negative.

Case 3

This boy is the twin of case 2; they were in the same amniotic sac and shared a common placenta. A 'peeling' rash on the face was observed when he was examined at the age of 15 weeks but this gradually disappeared. He had no arrhythmic problems and his ANF was negative when he was 2 years 9 months old.

Case 4

When 6 weeks old, this male infant was noted to have annular telangiectatic lesions with central atrophy and slight peripheral scaling on the forehead, scalp and above the left eye (Fig. 1). He

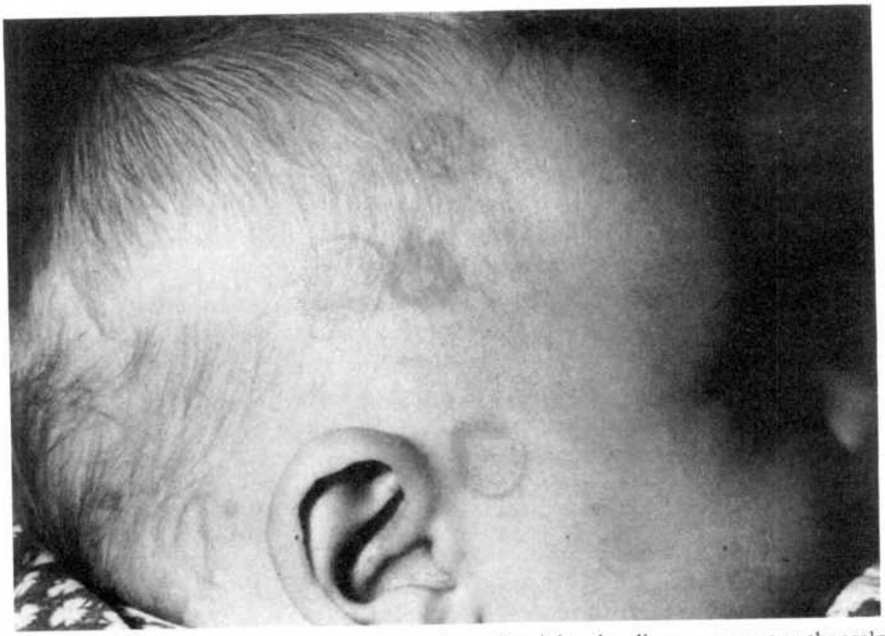

FIGURE 1. Case 4. Annular lesions with central atrophy and peripheral scaling are present on the scalp and face.

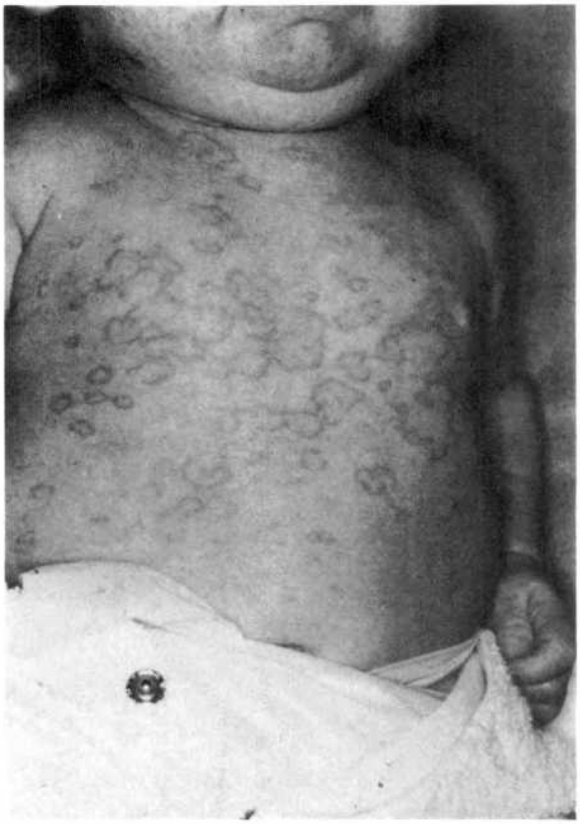

FIGURE 2. Case 5. Annular lesions, confluent in places and showing central atrophy, are present on the trunk.

was seen in the Dermatology Department and a diagnosis of neonatal LE was made. The lesions faded within the first 6 months of life without any therapy being given, leaving a trace of atrophy. The ANF test performed at the age of 4 months, was negative.

Case 5

This male infant was jaundiced at birth and received phototherapy with light of wavelength 400–475 nm during the neonatal period. When 3 weeks old he developed annular erythematous lesions on the trunk, which showed central pallor and atrophy (Fig. 2). Neonatal LE was diagnosed and by the age of 3 months, the lesions had almost completely disappeared without any treatment.

DISCUSSION

Neonatal LE with cutaneous features is, like its systemic counterpart, more frequent in females (Draznin *et al.*, 1979); only five males have been reported with the cutaneous syndrome (Reed, May & Tuffanelli, 1967; Miyagawa *et al.*, 1981; Lecha *et al.*, 1982). This female preponderance exists to a lesser extent in cases having congenital heart block, and of the sixty-seven infants summarized by Esscher & Scott (1979), forty-two were girls. Eight infants are described as showing both cardiac and cutaneous signs (McCue *et al.*, 1977; Winkler, Nora & Nora, 1977; Berube *et al.*, 1978; Esscher & Scott, 1979; Hardy *et al.*, 1979; Franco *et al.*, 1981; Weston *et al.*, 1982), but only one of these is male (Esscher & Scott, 1979). The explanation of this female preponderance in neonatal LE is unknown, but the observation suggests that there are infants at risk, perhaps mostly males, who do not develop the syndrome. Indeed Provost (1983) has shown this to be so by detecting the Ro antibody in an asymptomatic infant, suggesting that other factors, either genetic or environmental, are required for the manifestation of the condition.

Photosensitivity is a characteristic of subacute cutaneous LE and 62% of patients with this syndrome have Ro antibodies (Maddison, Provost & Reichlin, 1981). In the neonatal period, three of our patients received phototherapy with light of wavelength 400–475 nm: this could have been involved in producing their cutaneous lesions although the other infant (case 4) who did not receive this treatment still developed the eruption.

Neonatal LE may occur in siblings (Reed *et al.*, 1967; Esscher & Scott, 1979; Miyagawa *et al.*, 1981; Lee *et al.*, 1983) although it is unusual to see a combination of cutaneous and cardiac involvement within a sibship (Hull, Binns & Joyce, 1966; McCue *et al.*, 1977). Every pregnant woman with a history of connective tissue disorder should be screened for Ro and La antibodies to identify those at risk of producing infants with neonatal LE. These women and those who are asymptomatic but previously have had an affected infant, should be monitored during pregnancy for fetal bradycardia and their neonates carefully examined since congenital heart block is potentially fatal and usually permanent.

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Excess granulation tissue responses associated with isotretinoin therapy

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SUMMARY

Multiple polypoid projections of granulation tissue developed in two patients receiving isotretinoin for acne. Histological study of the lesions revealed increased amounts of non-sulphated acid mucopolysaccharides in the ground substance of the granulation tissue stroma. Complete resolution occurred following curettage with or without chemical cautery. The role of isotretinoin in the development of these lesions is discussed.

Oral isotretinoin (13-*cis*-retinoic acid) is effective in the treatment of recalcitrant cystic and conglobate acne, but reported clinical side-effects include cheilitis, conjunctivitis, xerosis, facial dermatitis, dryness of nasal and oral mucosa, epistaxis, hair loss, palmoplantar desquamation, arthralgias, decreased appetite, headache and fatigue (Jones *et al.*, 1983; Wagner & Plewig, 1980; Windhorst & Nigra, 1982). We now report the occurrence of multiple pyogenic granuloma-like lesions in two patients receiving isotretinoin, which appear to be similar to six previously published cases (Exner, Shamim & Pochi, 1983; Valentic, Barr & Weinstein, 1983; Campbell *et al.*, 1983).

CASE 1

A 27-year-old white male had exceedingly severe cystic acne of 14 years' duration, which responded poorly to conventional topical, intralesional, and oral therapy. Examination revealed multiple comedones, pustules, inflammatory nodules and scars involving the face, chest, back, upper arms and buttocks. Numerous sharply defined, irregularly shaped ulcers of 1–2·5 cm diameter with tightly adherent yellow-brown crusts and abscesses of 0·5–2 cm diameter were present on the chest, back and buttocks.

Oral isotretinoin (13-*cis*-retinoic acid) was started at a dosage of 80 mg orally twice daily (2·6

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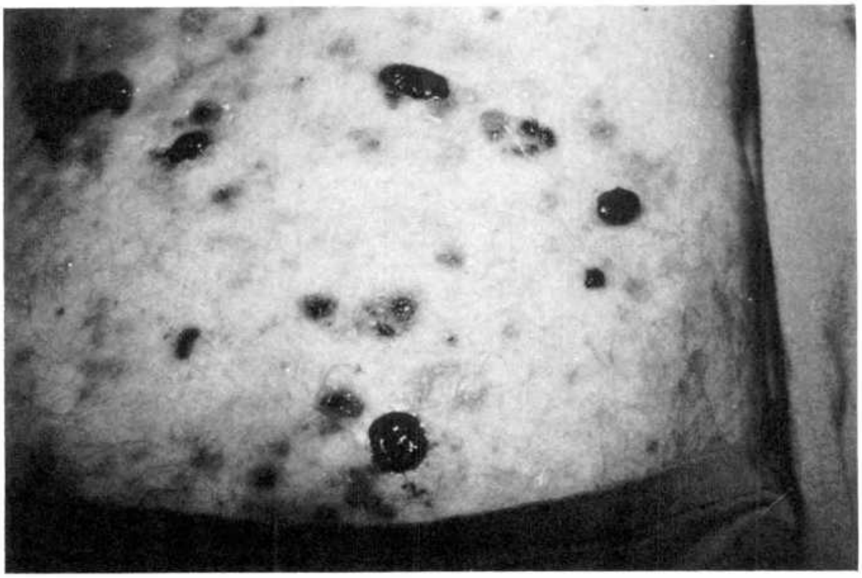

FIGURE 1. Right posterior flank demonstrating numerous glistening, polypoid areas of granulation tissue with acne scarring and post-inflammatory hyperpigmentation (case 1).

mg/kg/day). All systemic, intralesional and topical medicaments, with the exception of 5 mg of oral prednisone every morning and povidone–iodine baths twice daily, had been discontinued 2 weeks before starting isotretinoin. After 4 weeks of therapy, the previously noted areas of ulceration were moderately painful. When the now loosely adherent crusts were removed, a serosanguinous exudate was revealed. Following 8 weeks of therapy, the ulcers were unchanged in size and no longer covered with crusts. Instead, moist, friable granulation tissue was present in the floor of each ulcer. Ten weeks after the onset of treatment, the areas of previous ulceration demonstrated bright red, smooth, glistening, polypoid tissue (Fig. 1). The lesions were treated at 2-week intervals with curettage, followed by silver nitrate cautery. Most lesions resolved following one treatment and all had involuted following the third treatment. All lesions healed with erythematous, depressed, pliable, atrophic scars. Isotretinoin was discontinued after 16 weeks of therapy with marked improvement of all acne lesions.

The following laboratory studies performed prior to, during and following isotretinoin therapy were normal: RBC count; WBC count and differential; sedimentation rate; serum creatinine, BUN, total bilirubin, alkaline phosphatase, SGOT, LDH, cholesterol and triglycerides; urinalysis.

CASE 2

A 21-year-old white male had severe cystic acne of 7 years' duration, which was unresponsive to therapy. Examination revealed multiple comedones, cysts and inflammatory nodules distributed on the face, neck, upper chest and back. Isotretinoin was started at an initial dosage of 50 mg orally twice daily (1·3 mg/kg/day). After 6 weeks of therapy, a 1·5 × 2·0 cm moist, friable, exophytic lesion with a serous exudate developed on the posterior aspect of the patient's neck (Fig. 2). The lesion resolved without recurrence following a biopsy with curettage of the base.

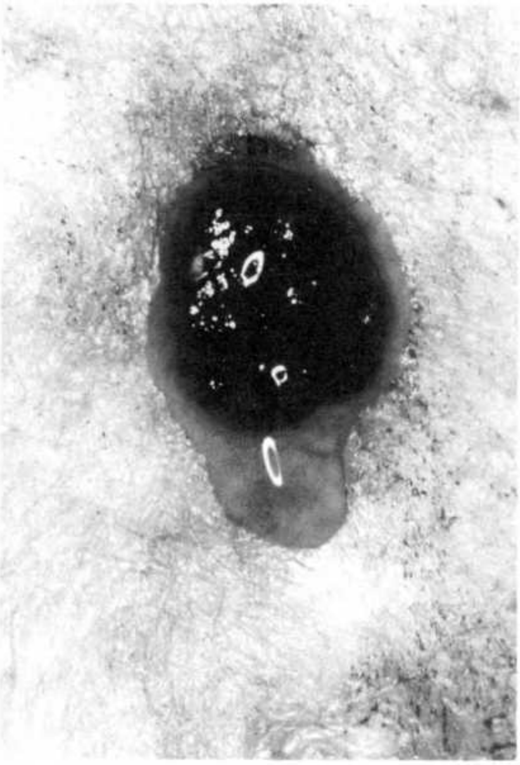

FIGURE 2. Posterior neck demonstrating exudative, exophytic, granulation tissue (case 2).

Twelve weeks into the treatment course, the dosage of isotretinoin was increased to 80 mg orally twice daily (2·1 mg/kg/day) in an attempt to clear his skin. At the end of 16 weeks of therapy, isotretinoin was discontinued, at which time his acne was markedly improved.

The following laboratory studies performed prior to and throughout therapy with isotretinoin were within normal limits: haematocrit, haemoglobin, RBC count; WBC count and differential; serum creatinine, BUN, total bilirubin, alkaline phosphatase, SGOT, LDH, cholesterol and triglycerides; urinalysis.

HISTOPATHOLOGY

Multiple biopsy specimens were obtained from representative lesions and stained with haematoxylin and eosin. In both patients, the epidermal layers were absent and the superficial dermis contained markedly oedematous, light blue staining stroma with numerous small blood vessels. A mixed perivascular inflammatory infiltrate with numerous plasma cells was present in the superficial and deep dermis (Fig. 3). The lower portion of the dermis demonstrated moderate fibroplasia with increased fibroblasts and collagenous fibres. In case 2, several clusters of multinucleated epithelioid cells of the foreign body type were noted in the superficial dermis (Fig. 4). No epidermal collarette at the base of the lesions was identified.

Additional sections were treated with Alcian blue, which stained the ground substance of the oedematous stroma blue at pH 2·5, but not at pH 0·5. Incubation of the sections with

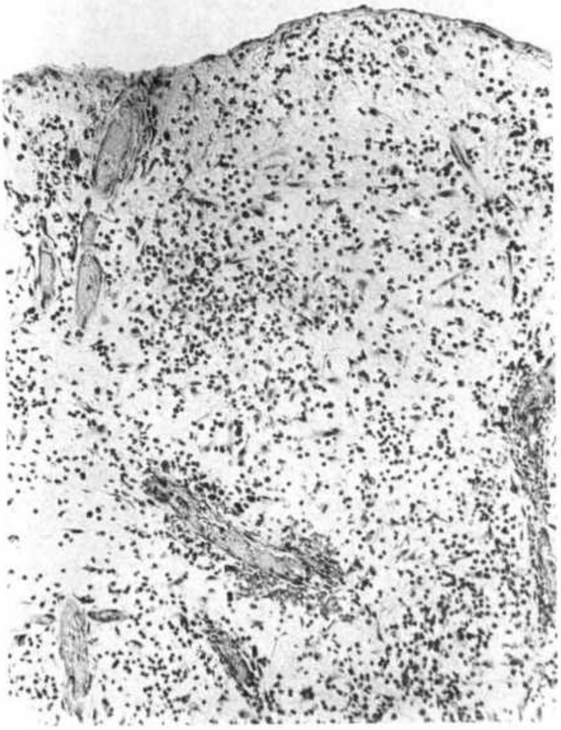

FIGURE 3. Photomicrograph of biopsy specimen showing absent epidermis with markedly oedematous superficial dermis containing a mixed perivascular inflammatory cell infiltrate and proliferative vessels (H & E, × 65).

hyaluronidase at 37°C for 1 h abolished most staining with Alcian blue, suggesting the presence of large amounts of non-sulphated acid mucopolysaccharides, including hyaluronic acid, in the ground substance. Periodic acid–Schiff stains were negative both on diastase-treated and non-treated sections, suggesting the absence of neutral mucopolysaccharide and glycogen. The presence of acid or neutral mucopolysaccharides was not demonstrated in adjacent normal dermis.

DISCUSSION

The exuberant granulation tissue noted in the cases under current consideration is similar to six previously published cases (Exner *et al.*, 1983; Valentic *et al.*, 1983; Campbell *et al.*, 1983). This reaction generally begins between the fourth and ninth week of treatment with isotretinoin. The excess granulation tissue develops at sites of both ulcerative and non-ulcerative cystic acne lesions. In our experience, all lesions responded to local therapy and discontinuation of isotretinoin was not necessary to effect resolution.

One explanation for the development of these lesions would be a stimulatory effect of isotretinoin on the process of wound healing. The ability of natural retinoids to promote wound healing has been described (Hunt, Andrews & Halliday, 1981; Seifer *et al.*, 1981). These cases

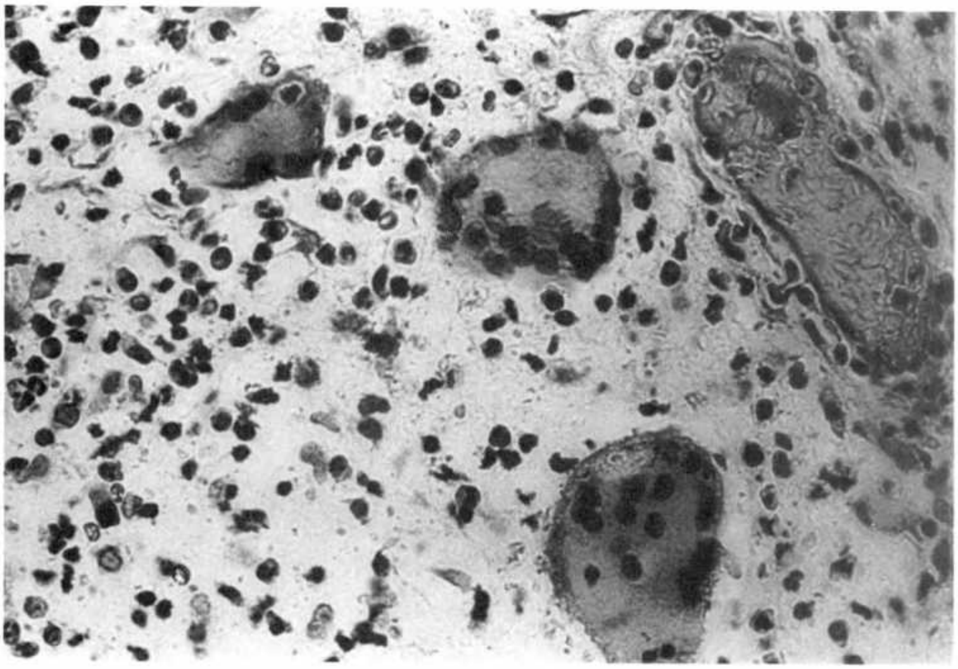

FIGURE 4. Three multinucleated epithelioid cells present in the superficial dermis near a small vessel (H & E, × 280).

may represent an exaggerated response of the healing process due to the administration of a synthetic retinoid. Similar lesions have been reported in patients receiving etretinate therapy (Williamson & Greenwood, 1983).

The presence of large amounts of acid mucopolysaccharides in the ground substance of the granulation tissue stroma may relate to the previously described role that retinoids play in the synthesis of glycoproteins (Adamo *et al.*, 1979; Elias & Williams, 1981; King & Tabiona, 1981; Nemanic, Fritsch & Elias, 1982) and acid mucopolysaccharides (Sporn *et al.*, 1976). Although most investigations regarding the effects of retinoids on the synthesis of glycoproteins have examined epithelial cells, dermal alterations following topical and oral tretinoin have been described (Mercier & Stuttgen, 1975). The alteration of the dermis in these cases may relate to the effects of retinoids on fibroblasts (Bauer, Seltzer & Eisen, 1982; Brinckerhoff *et al.*, 1982) with subsequent changes in both collagen and ground substance.

Future investigations should further delineate the effects of synthetic retinoids on human dermal connective tissue and wound healing. If patients on isotretinoin are prone to develop exuberant granulation tissue, caution may be advisable in performing surgical procedures, such as dermabrasion, in these individuals.

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Comment

MIGRATION AND MITOSIS IN THE EPIDERMIS

In a recent article, Mitrani (1983) asked the question 'Is upward basal cell movement independent of mitosis in the normal epidermis?', that is, whether migration is an active or passive process. Few would argue against mitosis and migration being linked. The question is how are they linked and which of them, mitosis or migration, comes first? Mitrani's paper presented computer simulations of the changes in the number of labelled cells and the total number of cells in the basal and spinous layers in unirradiated and irradiated epidermis. In spite of statements to the contrary, no *in vivo* data were presented. Instead the simulated curves were assessed in relation to data not shown, but published by Etoh, Taguchi and Tabachnick (1975). Since the paper quotes observations made by our group and contains some inconsistencies and, we believe, incorrect interpretations, we felt that some alternative considerations should be presented.

The paper states that cell kinetic data for mouse dorsal epidermis were taken from Etoh *et al.* (1975). This noteworthy paper, in fact, presented little or no cell kinetic data and reported studies only on guinea-pigs and not mice. Mitrani then continued to compare simulated data for mice with published data for guinea-pigs. This may not be a problem provided the proliferative populations behave in a similar fashion. The theoretical curves would be dependent not only on the average cell kinetic parameters including the total cell cycle, which was not mentioned and is difficult to assess accurately in any case, but also on the proliferative model assumed.

When the theoretical curves illustrated in the paper are in fact compared with all the available experimental data the picture is far from clear. Assuming that the basal layer contains about 100 cells/mm, the total cell numbers predicted by active and passive models (based on the assumptions for mouse skin presented Mitrani) can be compared with actual data from pigs and guinea-pigs (Fig. 1) and with data from mice (Fig. 2). Neither model fits the results for pig skin. The passive model appears to fit for guinea-pigs whereas the active model fits slightly better for the mouse data. Adoption of different assumptions will strongly influence the position and slope of the theoretical curves. This is particularly the case when the following are considered: (1) the number of cells/mm, (2) the cell cycle times, (3) the proliferative organization (the proportion of the proliferative cell population which function as stem cells), (4) whether or not there are maturing, non-dividing differentiated cells in the basal layer and (5) whether there is more than one kinetically distinct cell population, as well as the assumptions made concerning the way in which cells respond to radiation.

The author quotes some of our observations on the relative timing of compensatory mitosis after minor wounding (plucking) (Potten & Allen, 1975). We concluded, since the basal layer is very rapidly depleted of cells (which can only have moved into the suprabasal layers) and that this is *followed* and not preceded by compensatory mitosis, that mitosis is not required for this initial post-wounding migration. Similar conclusions have been reached from studies on other types of wounding (Krawezyk, 1971). Mitrani states that 'it is difficult to know how [our] conclusion was obtained since no data on migration rates was given'. It is true that migration rates were not presented but they are not needed. That many cells disappear from the basal layer is a quantitated observation and that this precedes any mitotic or DNA synthetic burst is the only point that was being made.

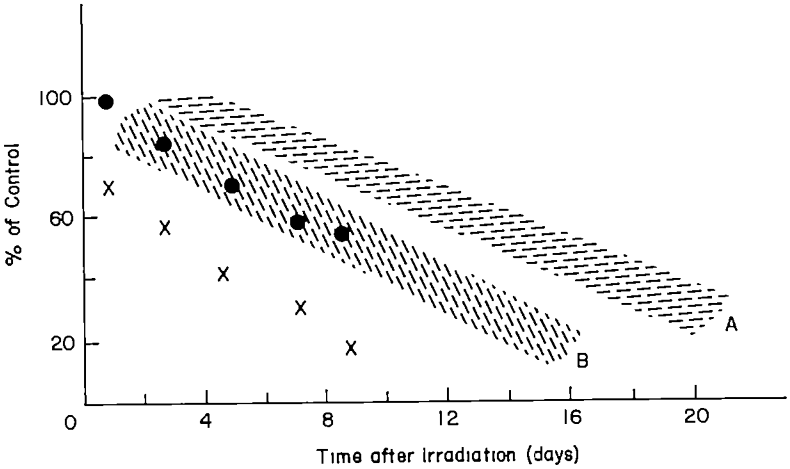

FIGURE 1. Changes in the number of basal cells per unit *length* of epidermis expressed as a percentage of control with time after various doses of radiation. No dose-dependence was observed and so the data from three sources have been pooled to give a general band of values that decreases at a rate of about 5% per day. The basal cell density has been expressed as a percentage of the value obtained in unirradiated animals (about 100 cells/mm). (A) Data for the epidermis from the pig (17-27Gy) (Archambeau *et al.*, 1979). (B) Data for the epidermis of the guinea-pig (22–50 Gy) (Etoh *et al.*, 1975; 1977) and rat tail (80–160 Gy) (de Rey & Klein-Szanto, 1972). (●) Passive migration model of Mitrani (1983). (×) Active migration model of Mitrani (1983).

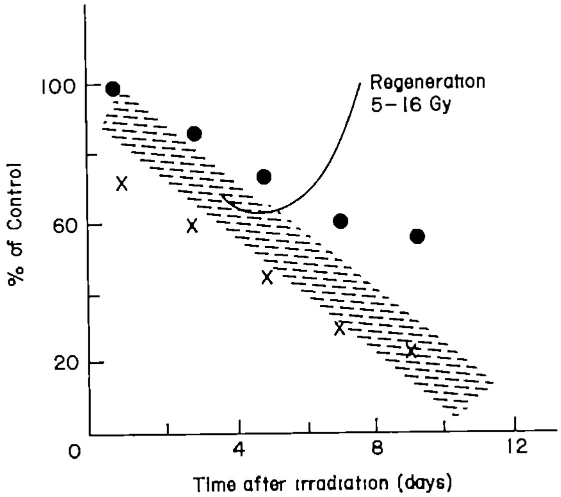

FIGURE 2. Changes in the number of basal cells per unit *area* of epidermis from mice expressed as a percentage of control with time after various doses of X-rays. No dose-dependence was observed and so the data have been pooled to give a general band of values decreasing at a rate of about 8% per day. The basal cell density has been expressed as a percentage of the value obtained in unirradiated animals (14,000 cells/mm^2). The data are from unpublished material of Al-Barwari (1978) which will appear in Potten (1984), involving both hairless and hairy mice and doses over the range 5–25 Gy. At doses below about 16 Gy compensatory regeneration can be observed. (●) Passive migration model of Mitrani (1983). (×) Active migration model of Mitrani (1983).

Theoretical simulations do little to resolve the question of the chicken and egg conundrum (which comes first, migration or mitosis?) (see Figs 1 and 2). There is at least some hard evidence in favour of the idea that migration does not need mitosis or precedes mitotic activity but as yet little or no actual data in support of the opposite view. The 'mitotic pressure' hypothesis was assumed in many early reports largely as a matter of convenience and has become part of dermatological belief without the support of hard evidence. The classic paper quoted by Mitrani as providing 'supporting evidence' in actual fact makes the statement that the *oesophageal* basal layer 'appears crowded and (hence) it is reasonable to assume that this layer is saturated with cells. Hence, if more cells are added by mitotic division, they can be accommodated only by forcing out an equivalent number of cells' (Leblond, Greulich & Pereira, 1964). This does not constitute supporting evidence, however.

One simple and yet obvious problem with the mitotic pressure hypothesis as applied to the epidermis on the back of a mouse is that the basal cells do not present an appearance consistent with being 'under pressure' (Fig. 3) [see Potten (1981) which also provides electron microscope pictures of these thin areas]. The basal cells are in many places very thin, extended cells with large regions of thin cytoplasm. Secondly, epidermal cells can clearly move as independent individuals in culture and also *in vivo* during the course of healing a wound. Increased mitotic activity in wounds usually occurs only *after* significant cell migration across a wound and inhibitors of mitotic activity do not influence the movement of cells in blister wounds (Krawcyzk, 1971).

When human keratinocytes are induced to grow as a monolayer in culture by reducing the calcium levels in the medium, differentiated cells still synthesize involucrin, a protein normally associated with suprabasal cells of the epidermis. When the calcium levels are raised these differentiated, involucrin-containing cells move into the next layer within 24 h (i.e. stratification is induced) (Watt & Green, 1982). This migration does not apparently require protein or glycoprotein synthesis nor does it require an increase in mitotic activity. Furthermore, it continues when mitosis is inhibited. In fact, the movement continued in the presence of cycloheximide, tunicamycin or cytosine arabinoside (Watt, 1984).

Finally, in other systems, notably the mucosa of the small intestine where the columnar cells certainly present an appearance suggesting that they are density packed, there is an extensive literature indicating that migration of cells on to the villus continues when mitotic activity is suppressed, the cell density is decreased or cell production on to the villi is interrupted (Hughes *et al.*, 1958; McGrath & Congdon, 1959; McGrath, 1960; Sherman & Quastler, 1960; Galjaard & Bootsma, 1969; Altmann, 1974), although at least some of this migration may be attributable to cell enlargement (see McGrath, 1960). In fact, from recent, as yet unpublished, data of Kaur it is clear that: (1) migration of cells from the crypt continues not only after large, single doses of radiation but after highly destructive cocktails of cytotoxic drugs; (2) probably two mechanisms are involved, one an active process out of the crypts involving protein synthesis, since protein synthesis inhibitors can stop this migration (see also Altmann, 1975), and the second a passive process once the cells reach the villus that may in some way be driven by the rhythmic contractions of the intestine and its villi.

In the filiform papillae of mouse tongue, basal cells are aligned on an undulating basement membrane in a pattern according to their proliferative potential; post-mitotic cells and those with least capacity for division are more superficially placed on the connective tissue papillae (Hume & Potten, 1980). Conversely, the proportion of proliferating cells increases with decreasing basal cell position (the stem cell zone, cell position 1, is the lowest ring of cells on the connective tissue). In this system there are two distinct patterns of cell migration as detected by following the movement of labelled cells: (1) along the basement membrane towards the apex of the connective tissue papillae from where they move suprabasally into the overlying column of cells; (2) suprabasally, mainly from low cell positions. Because of the rapidity of migration and cell cycle times in this system it is difficult to imagine mitotic cells actively pushing large numbers of cells along the basement membrane in an orderly fashion, especially as the mechanics of the system dictate that the rate of migration must increase with increasing basal cell position on the connective tissue papillae, yet the proportion of dividing cells is falling. It is, however, possible that migration, directly suprabasally, from low cell positions could be the result of 'mitotic pressure' although here the situation is less clear (Hume, 1983).

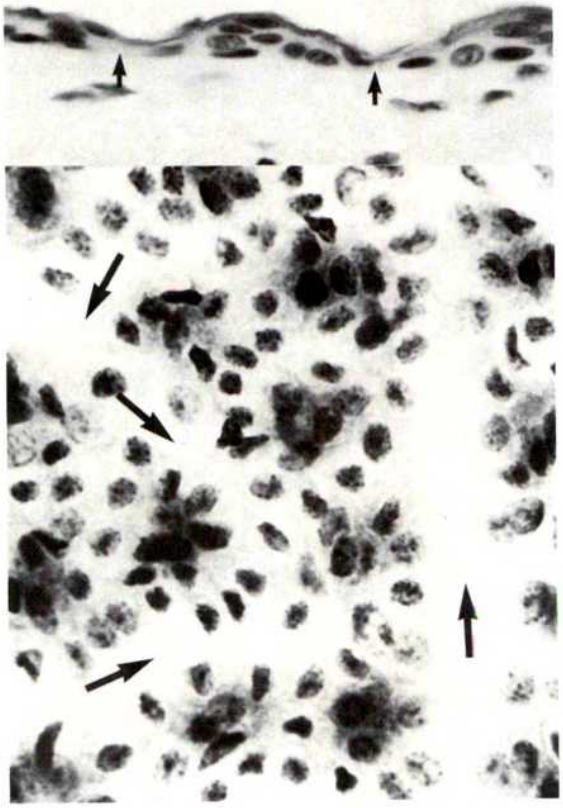

FIGURE 3. Spatial distribution of basal cells in the epidermis from the back of a mouse. The epidermis has been separated from the dermis (lower picture). The dark clusters result in part from the fact that the suprabasal layers, through which the light is focussed, are all aligned into the columns and the cell nuclei, which are stained with haematoxylin, form a cluster which is largely out of focus above another cluster of basal nuclei. There are clear areas where there are few nuclei (arrows) and an uneven general distribution of cell nuclei. Evidence of this unevenness can also clearly be seen in paraffin sections (upper picture) through the epidermis (arrows). (× 600.)

Thus, the situation remains uncertain but on balance the evidence would seem to favour a situation where migration precedes mitosis (i.e. is not driven by mitotic activity). The computer simulations presented by Mitrani on the whole do not help to resolve the issue.

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Society Proceedings

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(Formerly the Investigative Group of the British Association of Dermatologists)

PAPERS

An animal model for measuring the UV-A protection provided by topical sunscreens

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The efficacy of UV-A sunscreens is difficult to assess. We have used a photosensitized animal in which the reaction is UV-A dependent. Contact photosensitivity (CPS) in BALB/c mice was induced on trunk skin by tetrachlorsalicylanilide (TCSA) and UV-A. Seven days later the degree of CPS was quantified by measuring the increase in ear thickness after elicitation with TCSA and UV-A. Sensitized animals showed an increase in ear thickness (in cm × 10^{-3}) of 18·64 ± 4·21 (s.d.), significantly greater ($P < 0{\cdot}0005$) than non-sensitized animals (1·81 ± 1·00).

UV-A sunscreens should suppress this reaction. Elicitation is performed on the ears with the sunscreen being applied to one ear before exposure to UV-A. The following products have been assessed in separate groups of animals: (A) PABA ester 6% and oxybenzone 5% in a cream base; (B) the base of product A; (C) PABA 5% in ethanol; (D) the base of product C; (E) PABA ester 1·4% in a cream base. Mean increase in ear thickness was: (A) 1·38 ± 0·92; (B) 19·5 ± 2·05; (C) 7·08 ± 3·64; (D) 8·75 ± 4·37; (E) 26·5 ± 2·81. Results were compared with the photosensitized controls that received no sunscreen (18·64 ± 4·21). There was a highly significant suppression of ear swelling by Product A when compared with the control ($P < 0{\cdot}0005$). The base, B, gave no suppression. Product C gave some suppression but this was no different from its base D ($P > 0{\cdot}3$). Product E resulted in an increase in ear thickness that was significantly greater than the control ($P < 0{\cdot}0025$).

These preliminary results show that this animal model can be used to quantify the protection offered against UV-A by a topical sunscreen containing oxybenzone (product A). Sunscreens containing PABA alone (products C and E) would not be expected to protect against UV-A and they failed to do so in this model. PABA in ethanol did produce some suppression of ear swelling but it was no greater than the ethanol alone. It is possible that ethanol interferes with the test system and this will require further investigation.

Possible inhibitory mechanisms for contact sensitization by DNCB, following UV-B induced damage to Langerhans cells

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In experimental animals, UV-B damages epidermal Langerhans cells (ELC). Induction of subsequent contact sensitivity is impaired because immune suppressor mechanisms (tolerance) are activated. We have studied people receiving UV-B therapy for psoriasis to see whether similar effects occur. Epidermal suction biopsies were taken from twenty-two psoriatic subjects at different times during UV-B therapy. ELC were stained for ATPase and their numbers per mm² counted. After ten treatments (2 weeks) there was a 65% reduction, from 720/mm² (s.e. = 60) to 250/mm² (s.e. = 30). They remained at about 50% throughout the treatment period. To examine the functional consequences of this damage to ELC we measured contact sensitization by dinitrochlorobenzene (DNCB). The 100% sensitizing dose, 500 μg, was applied to twenty-four subjects after 3 weeks of UV-B treatment. Four weeks after the completion of treatment, sensitivity was assessed by measurement of responses to challenge with four doses of DNCB (from 25 μg to 3·125 μg) applied simultaneously. Change in skin fold thickness was measured at each challenge site at 48 h, and dose-response curves were plotted. Forty-four per cent of subjects showed no detectable reactivity, and those who did gave significantly reduced responses compared with untreated controls. If the failure to respond were due to inhibitory mechanisms such as tolerance, normal reactivity should not occur upon boosting with more DNCB. Therefore, since the first challenge should have a boosting effect, unresponsive subjects were given a second challenge 4 weeks later, and responses compared with those from control groups set up to assess boosting effects. The UV-B treated subjects showed less boosting than controls which suggests the presence of mechanisms akin to the tolerance seen in experimental animals.

The action of topical steroids, dithranol, and PUVA on T lymphocytes and dendritic cells in psoriasis

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Eighteen patients with psoriasis were divided into three groups; one was treated with topical steroids, another with PUVA and a third with dithranol. Serial biopsies were taken before treatment and then at 2, 4, 7, 14, 21 and 28 days. Using monoclonal antibodies the number of dendritic, TH, and TS cells in the epidermis and the DR expression of these cells were determined, per 50 high power fields in each biopsy. In patients treated with dithranol there was no decrease in the total number of dendritic cells or the number of DR$^+$ dendritic cells. However, in those treated with topical steroids a significant decrease was apparent at 4 days for both total and DR$^+$ dendritic cells with further fall up to 14 days. In patients treated with PUVA there was an increase in the number of both dendritic and DR$^+$ dendritic cells during the first week, but then a gradual fall to significantly lower numbers by 4 weeks. A significant fall in TH and DR$^+$ TH cells was apparent after 2 days in the topical steroid group, 14 days in the PUVA group and at 7 days in the dithranol group. Similar results were obtained for TS and DR$^+$ TS cells. These results show in all treatments a fall in the total number of TH and TS cells and the numbers showing DR$^+$ expression, and this decrease precedes the fall that occurs in dendritic cells with topical steroids and PUVA treatment, but no fall occurs in these cells with dithranol. The speed of fall in the number of T lymphocytes correlates with the speed of clinical improvement with these different therapies. If these treatments clear psoriasis via interference with an immunological pathway their primary site of action appears to be on the lymphocytes.

UV-B induced collagen changes in hairless mouse skin

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After irradiation with UV-B (280–315 nm), the Skh 1 (hairless albino) mouse shows histological features similar to those of solar elastosis in man (Kligman *et al.*, *J. Invest. Derm.* 1982, **78**, 181). These observations are difficult to quantify, thus we have used biochemical techniques to measure UV-B induced changes in dermal collagen.

Groups of ten Skh 1 mice were irradiated dorsally with a daily dose of 100 mJ/cm^2 UV-B (Westinghouse FS20 Sunlamps) for 12, 24, 30 and 36 weeks. This treatment was highly tumorigenic in the latter three groups. Non-irradiated controls were housed under identical conditions. Additional groups were irradiated for similar periods but kept for 6 to 24 weeks, without irradiation, before collagen analysis. Samples of pelt were taken from dorsal and ventral surfaces and types I and III collagen were quantified densitometrically after cyanogen bromide digestion and polyacrylamide gel electrophoresis. Type III collagen was expressed as (III)/[(III) + (I)] × 100% and the ratio of dorsal/ventral type III was determined for each mouse.

This type III ratio increased by about 30% ($P < 0{\cdot}01$) in all irradiated groups when compared with controls. However, mice left for a period between the end of the irradiation schedule and collagen analysis showed a tendency for recovery to control levels. Biochemical changes were detected before elastosis was histologically apparent.

These data are in agreement with those of our previous human studies which showed an increase in type III collagen in solar exposed skin when compared to non-exposed sites. Thus they support the use of the mouse model for further photo-ageing investigations.

Evidence for a novel vasoactive mechanism in chronic idiopathic urticaria (CIU)

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In a double-blind study thirty patients with CIU were randomly divided into two groups. For week 1 both received placebo, for weeks 2–7 astemizole 1 mg tds or chlorpheniramine 4 mg tds, followed by 4 weeks placebo. Itch (10 cm) and wealing were graded daily by the patients, and weals were counted by the physician at the end of each treatment and grouped on a 0–4 scale. Histamine wealing was measured before and at the end of active treatment in eight patients randomly selected from each group. Both drugs reduced the severity and duration of itch ($P < 0{\cdot}0001$ for astemizole and chlorpheniramine), the reduction being significantly greater with astemizole than chlorpheniramine. Weal number decreased comparably from $3{\cdot}1 \pm 0{\cdot}06$ and $3{\cdot}0 \pm 0{\cdot}09$ to $1{\cdot}6 \pm 0{\cdot}06$ and $1{\cdot}6 \pm 0{\cdot}04$ for astemizole and chlorpheniramine ($P < 0{\cdot}0001$) and so did weal duration ($P < 0{\cdot}001$ for astemizole, $P < 0{\cdot}003$ for chlorpheniramine). Although there was no significant difference in effectiveness of both drugs, weal dose-responses showed that chlorpheniramine reduced histamine weal potency by only 28% whilst astemizole reduced histamine potency by >98%. Since the patients continued to develop urticarial weals despite the virtually complete inhibition of histamine wealing by astemizole it is concluded that in addition to H1 active histamine, a vasoactive mechanism, as yet unknown, is involved in chronic idiopathic urticaria. This explains the poor correlation between the H1 receptor antagonistic potency of antihistamines and their therapeutic effect.

Skin monohydroxy fatty acids: correlation of biosynthesis with proinflammatory activity

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Several monohydroxy fatty acids have been identified in psoriatic lesions (Camp *et al.*, *Prostaglandins* 1983, **26**, 431). In order to determine the relevance of these compounds to the inflammatory changes in psoriasis, the pro-inflammatory properties of nine unsaturated fatty acids and, where indicated, their monohydroxy metabolites, were determined in human skin. Test compounds were applied in ethanol, which was evaporated and the residues occluded for 6 h. The areas of erythema were then measured and their intensities scored. The relative amounts of the positional monohydroxy isomers of the fatty acids were also determined in psoriatic scale by a novel gas chromatography–mass spectrometric technique.

Of the fatty acids tested, only arachidonic acid (AA) and eicosapentaenoic acid (EPA) gave measurable inflammatory reactions at doses of 250 μg ($n=5$). Topical application of the positional monohydroxy isomers of these two fatty acids (1–100 μg; $n=5$) showed that the 12-hydroxy metabolite of both compounds was the most potent in causing erythema. Quantification of the positional monohydroxy isomers of AA and EPA in scale samples from seven patients showed that the 12-hydroxy metabolites were the most abundant in both cases, although the AA metabolite was present in approximately 100-fold greater amounts than the EPA metabolite.

These results suggest that human skin lipoxygenase enzymes are most active in forming, from AA and EPA, those monohydroxy isomers with the greatest pro-inflammatory effects in skin. However, in psoriasis, monohydroxy metabolites of AA appear to be of greater potential importance than those of EPA in mediating inflammation, in view of the relative amounts present.

The effects of 13-*cis* retinoic acid on hepatic and cutaneous mono-oxygenase activities: possible cancer-protective mechanism

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The mechanism of the cancer-protective effect of retinoids is unknown. A major factor involved in the development of neoplasia is the metabolic activation of chemical carcinogens to reactive metabolites by the microsomal mono-oxygenase system. We have, therefore, investigated the effect of 13-*cis* RA on hepatic and cutaneous microsomal mono-oxygenase activities, responsible for the metabolism of carcinogens, using aryl hydrocarbon hydroxylase (AHH), ethoxycoumarin dealkylase (EOD) and ethoxyresorufin dealkylase (ERD) as indicators of mono-oxygenation. IP administration of 13-*cis* RA (0·3 μmol/kg) to adult male hairless mice decreased basal levels of hepatic EOD and AHH by 20% while decreasing ERD by 70%; cutaneous EOD and AHH were similarly reduced by 20 and 24% respectively, and ERD by 70%. In control mice induction of hepatic mono-oxygenase by the carcinogen 3-methyl cholanthrene (3MC) increased EOD and AHH fourfold and ERD eightfold whereas in mice pretreated with 13-*cis*, MC induced hepatic AHH and EOD threefold and ERD only twofold.

Similar results were obtained with cutaneous mono-oxygenases. In control mice topical 3MC induced cutaneous AHH and EOD fivefold and ERD tenfold, whereas in mice pretreated with 13-*cis* cutaneous EOD and AHH were increased fourfold and ERD twofold. The effects of 13-*cis* are therefore relatively specific to ERD induction by polycyclic hydrocarbons. Since of the three mono-oxygenase activities measured ERD has been shown to be a relatively specific indicator of the production of mutagenic metabolites from polycyclic hydrocarbons, the findings suggest that one mechanism of cancer protection by 13-*cis* may be a reduction in the formation of reactive metabolites.

Topical indomethacin does not inhibit anthralin irritancy

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Topical indomethacin gel (1%) and gel base were compared in a single blind study of their effect on anthralin irritancy. Anthralin (10 or 100 μg) dissolved in chloroform, was applied to clinically normal forearm skin of patients with psoriasis and the chloroform allowed to evaporate off. Irritancy was measured by the increase in skin fold thickness 48 h later using Harpenden calipers. Using this method anthralin irritancy is maximal at 42 h. Four sets of experiments were done in which indomethacin gel was applied; at the onset of erythema, i.e. 18 h and 6 h thereafter, immediately after the application of anthralin and the occlusion of the site, during different phases of the irritant reaction, and at various intervals before anthralin application. In none of these studies was there any difference between the active and placebo sites. To test the efficacy of the indomethacin gel, active and placebo gel was applied to forearm skin of five subjects immediately after exposure to solar-simulating radiation (45 s, 112·5 mJ cm^{-2}). There was a significant reduction in erythema, measured using a reflectance erythrometer, in each subject on the indomethacin gel-treated site. We conclude that application of a topical preparation of indomethacin which is able to reduce a prostaglandin-associated reaction of skin, has no effect on anthralin irritancy.

Paradoxes and pitfalls in the interpretation of plasma 5-S-cysteinyldopa levels

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5-S-cysteinyldopa (5-S-CD) arises as an intermediate in melanin synthesis, produced by the action of the enzyme tyrosinase on the amino acid tyrosine. 5-S-CD is present in urine and plasma, and levels may be raised in metastatic melanoma. Having studied the use of this dipeptide as a marker for melanoma, we draw attention to factors which may affect the interpretation of results, and to certain unexpected findings which arose in our studies.

Plasma 5-S-CD was determined by high-pressure liquid chromatography with electrochemical detection, using 5-S-D-cysteinyl-L-dopa as internal standard.

Study of healthy volunteers established a figure of 20 nmol/l as the upper limit of normal plasma 5-S-CD concentrations. Increased levels were seen in some patients with extensive melanoma, but were also noted in patients with chronic renal failure and in patients being treated with carbidopa and L-dopa for Parkinson's disease, thus indicating possible sources of false positive results. Another limitation to the use of 5-S-CD measurements was seen in the number of patients with known extensive melanoma and normal 5-S-CD levels.

An unexpected finding in our studies was the observation that patients with tyrosinase-negative oculocutaneous albinism had normal plasma 5-S-CD levels, despite the lack of active tyrosinase in these patients.

We conclude that factors other than tumour load must be taken into account when interpreting the plasma levels of 5-S-CD in patients with melanoma, and that synthesis of 5-S-CD does not necessarily depend on the presence of tyrosinase in the melanocytes.

Structural and functional properties of the dermo-epidermal junction in obligate heterozygotes for recessive forms of epidermolysis bullosa

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The possibility that obligate heterozygotes for the severe recessive forms of epidermolysis bullosa (EB) may partially manifest the structural and functional abnormalities of the dermo-epidermal junction present in sufferers was investigated. A controlled morphometric study of the junction was undertaken in four parents of children with severe recessive dystrophic EB (RDEB), which is characterized by an absence of anchoring fibrils, and in five heterozygotes for severe junctional EB (JEB), which is associated with hemidesmosomes lacking normal sub-basal dense plates (SBDP). In addition, suction blister times (SBT), which are markedly reduced in both RDEB and JEB, were measured on the abdomen and limbs at a suction pressure of 200 mmHg below atmospheric, in five heterozygotes for RDEB and in thirteen carriers of JEB.

Compared with normal controls, anchoring fibril numbers were reduced in skin from RDEB heterozygotes ($P < 0{\cdot}05$), although three of the four counts fell within the normal range. JEB heterozygotes possessed numerically normal hemidesmosomes with normal SBDP. In RDEB heterozygotes, SBT were not reduced compared with thirteen control subjects. In JEB heterozygotes the mean SBT were normal in three of the tested regions, but blistering occurred more rapidly on the upper arm ($P < 0{\cdot}05$), and furthermore, two of the thirteen subjects blistered abnormally quickly in most body regions.

In conclusion, the anchoring fibril deficiency in RDEB and the rapid suction blister formation in JEB may be partially expressed in the heterozygotes for these conditions. However, because of the wide normal variation in these two parameters, the changes present in the carrier states are probably too small for use in genetic screening.

Arachidonic acid metabolism by peripheral blood cells in psoriasis

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We have investigated the metabolism of arachidonic acid by mixed peripheral blood cells in twenty patients with psoriasis and twenty-one controls. EDTA-anticoagulated blood was mixed with 6% dextran to sediment the red cells, and the resulting cell suspension was washed twice in Dulbecco's buffer. The volume of the final suspension was adjusted so that the white cell count was $1{\cdot}5 \times 10^7$ ml^{-1} in every case; a manual platelet count was also done. The suspension was incubated on 0·5 ml volumes with 0·1 μCi [^{14}C] arachidonic acid for 5 min at 37°C, the reaction terminated by acidification to pH 3, and the samples extracted twice with ethyl acetate. The residue was analysed by thin-layer chromatography and the results expressed as the percentage of the total recovered radioactivity in each product.

Both the whole blood and mixed cell suspensions in psoriatics had higher platelet counts than in the controls ($P = 0{\cdot}001$). Conversion of substrate to TXB_2 by psoriatic platelets was reduced ($P = 0{\cdot}001$) but there did not appear to be any significant difference in the production of 12-HETE in the two populations.

The major white cell product of arachidonic acid metabolism was expected to be LTB_4, but the peak obtained did not quite chromatograph with the LTB_4 standard and its identity is currently being determined. It is most likely to be a lipoxygenase product, being removed by BW755C but not by indomethacin and is thought to be 12,20-diHETE. It was produced in significantly increased amounts by the psoriatic mixed cells compared with the controls ($P = 0{\cdot}02$), and was unaffected by the number of platelets present.

The results of this study add further support to the concept that arachidonic acid metabolism is abnormal in psoriasis. Further studies are in hand to determine whether these changes are primary or secondary to extensive skin disease.

Determination of platelet activating factor in psoriasis

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Platelet activating factor (PAF) is a mixture of homologous phosphatidyl choline ethers with the characteristics of a mediator of inflammation. In view of its chemo-attractant properties towards neutrophils we have been interested in measuring this substance in psoriatic skin and have reported the isolation, by chromatographic means, of biologically active PAF-like material from psoriatic scale (Mallet *et al.*, *J. Chromatogr.* 1984, **309**, 160). This work has now been extended to achieve the full characterization of the mixture of these ether phospholipids.

The methods employed are based upon mass spectrometric techniques (Satouchi *et al.*, *J. Biochem. Japan* 1983, **94**, 2067) and on improved high-performance liquid chromatography procedures.

The PAF we isolated from psoriatic scale contained a mixture of the saturated C-16 and C-18 homologues in the molar proportions 3 : 1 and no detectable level of any PAF containing unsaturated alkyl groups was observed.

The sensitivity and specificity of this method would appear to open the way to determining the relevance of this material in the pathogenesis of psoriasis and of other inflammatory diseases.

The relationship of α-keratins in dyskeratotic stratum corneum to the normal keratinization pathway

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Previous studies reporting abnormal tonofilament chain (α-keratin) compositions in the stratum corneum of a number of dyskeratoses have been purely descriptive. The α-keratin compositions of normal keratinocytes at all stages of differentiation are now known, and the purpose of the present study was to interpret abnormal stratum corneum compositions in terms of their position on, or deviation from, the normal keratinization pathway.

In each of the fifteen conditions studied, the stratum corneum had an abnormal α-keratin composition which was identical to that seen in normal basal, spinous or granular cells, i.e. on the normal pathway of keratinization.

The stratum corneum α-keratin composition was not disease-specific, although certain pairs of conditions (e.g. psoriasis and pityriasis rubra pilaris) could be distinguished diagnostically. The majority of conditions contained spinous cell-like α-keratins, similar to those observed in the stratum corneum of normal epidermis after experimentally induced hyperproliferation. In these conditions it therefore appears that incomplete α-keratin modifications are a consequence of epidermal hyperproliferation.

The heaviest (M_r 70 Kd) α-keratin present in normal stratum corneum is also detected in the stratum corneum of a number of hyperproliferative skin disorders and in the stratum corneum of normal epidermis after experimentally induced hyperproliferation. Psoriatic stratum corneum differs from that of all other conditions studied in that the 70 Kd α-keratin is consistently either decreased or absent. This suggests that in psoriasis, there is an altered pathway of keratinization rather than incomplete α-keratin modification as a secondary effect of hyperproliferation.

Glycoprotein and glycosaminoglycan synthesis in cultured human keratinocytes

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The biosynthesis of glycoproteins and glycosaminoglycans by human keratinocytes (HK) cultured on 3T3 feeder layers has been examined by metabolic labelling with [^{3}H]glucosamine and SDS polyacrylamide gel electrophoresis.

HK synthesized a complex pattern of glycosaminoglycans and glycoproteins. Major labelled glycoproteins in the cell layer mol. wt. 165K to 130K were identified as cell-surface sialoglycoproteins by their susceptibility to trypsin and by comparison with the plasma membrane glycoproteins previously isolated and characterized from pig ear epidermis. A significant amount of [^{3}H]glucosamine-labelled material was recovered from the culture medium of HK. The synthesis of extracellular glycosaminoglycan (mainly hyaluronate) was predicted from previous studies of pig epidermal explant cultures. However, HK also released a number of glycoproteins into the culture medium. These components were generally of lower mol. wt. than the major membrane glycoproteins and appeared similar to the 'soluble' glycoproteins previously characterized in pig epidermis.

Comparison with the glycoproteins synthesized by other cells cultured from human skin suggested that the major labelled glycoproteins in the cell layer and medium of HK were not derived from contaminating fibroblasts, melanocytes or 3T3 feeder cells.

HK grown in low Ca^{2+} medium and then switched to 1 mM Ca^{2+} increased their incorporation of [^{3}H]glucosamine into glycoproteins two- to fivefold. This effect was specific for glycoproteins since glycosaminoglycan synthesis was unaffected by the change in Ca^{2+} concentration. In contrast, HK grown in vitamin A-depleted medium synthesized considerably reduced levels of glycosaminoglycan and this was increased by the addition of 10^{-6} M all-*trans* retinoic acid. Glycoprotein synthesis was unaffected by low concentrations of retinoids.

Characterization of keratinocyte blistering *in vitro*

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Blisters have been observed in cell cultures of normal epidermis (Fuchs & Green, *Cell* 1981, **25**, 617) and of epidermis from patients with epidermolysis bullosa (Leigh *et al.*, *Br. J. Dermatol.* 1983, **108**, 233). Our aim was to further characterize the blistering process in cell cultures of normal human epidermis.

Keratinocytes were grown in 35 mm collagen-coated dishes with a 3T3 feeder layer. Blisters were macroscopic, fluid-filled structures 0·5–2 mm high and 0·5–5 mm in diameter which remained for several days before collapsing. Blistering commenced around day 12, prior to confluency, and new blisters were formed for up to 5 weeks post-plating. Maximal numbers (up to 70 per dish) were present around days 12–20. Blisters arose via a cleavage at the cell/dish interface to form a roof composed of 6–9 cell layers. The lowest cell layer appeared metabolically active and possessed a well-developed endoplasmic reticulum, Golgi and mitochondria, but, in contrast to peri-blister regions, lacked hemidesmosomes. The central 2–3 layers contained membrane-coating granules and prominent keratohyalin granules while the superficial strata resembled rudimentary corneocytes. Blisters formed irrespective of donor site or donor age, and were noted on various alternative substrates (collagen, 3T3 + plastic, plastic alone). The timing of initial blistering was not obviously affected by seeding density (10^4–10^6 per dish) or degree of confluency but was probably related to culture age and possibly, therefore, differentiation.

Cultures supplemented with 10^{-5} M vitamin A formed no blisters after 25 days and displayed only a few small keratohyalin granules. Ouabain (10^{-7} M), an inhibitor of active transport, caused blister collapse and a reversible inhibition of new blister formation.

We conclude that blisters are a consistent finding in keratinocyte cultures grown under standard conditions. Their formation may be associated with culture age and active transport triggered during differentiation.

Epidermal changes in skin treated with all-*trans* retinoic acid and Motretinide

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The retinoids exert a profound effect on epidermal differentiation and improve patients with a variety of skin diseases in which disordered differentiation plays a pathogenetic role. The mode of action of these drugs is uncertain and it is not even clear whether topically applied retinoids have the same type of effects as when these drugs are administered systemically. In this study we have compared the effects of topical all-*trans* retinoid acid (RA) and Motretinide (Mo) with unmedicated vehicle in twelve normal subjects and twelve patients with ichthyosis over a 21-day treatment period. Stratum corneum (SC) shedding was increased by both topical retinoids in normal skin compared to controls when assessed by a dansyl chloride fluorescence technique. Topical Mo also significantly increased the rate of SC shedding in patients with ichthyosis. Tritiated thymidine autoradiographic labelling indices were elevated in RA and Mo treated skin in both normal and ichthyotic subjects but this only reached statistical significance in RA treated ichthyotic skin. Cytochemical studies demonstrated alterations in non-specific esterase and succinic dehydrogenase activities in ichthyotic skin in RA and Mo treated areas compared with control, but no change in these parameters in normal subjects. Epidermal thickness increased in normal and ichthyotic skin after RA and Mo treatment and this was most evident in RA-treated normal skin. Previous results of our studies using systemically administered etretinate and an arotinoid have demonstrated similar findings to those reported here in some parameters but different results in others. We conclude that (a) topically applied Mo has similar, but not as marked epidermal effects as RA, and (b) that the changes observed after retinoid administration depend on (i) the particular retinoid administered, (ii) the route of administration.

Changes in lipid composition during epidermal maturation: comparisons between consecutive sections through pig epidermis

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It is generally accepted that lipids are involved in the formation of the epidermal permeability barrier, and results from histochemical and biochemical examination support this hypothesis. However, the only attempts to quantitate lipids in different strata of epidermis have involved aqueous enzyme separation, which can extract polar compounds. To overcome this, we froze pig skin by pressing its surface onto a cooled copper block (−70°C) and then cut twelve successive horizontal 8 μm sections through the epidermis from the surface. Ten samples from each of five pigs were analysed by thin-layer chromatography for nineteen lipids.

The concentrations of the five phospholipids examined were relatively constant in the deeper layers with a sudden decrease at the stratum corneum. Cholesteryl sulphate and the glucosyl ceramides, especially acylglucosylceramide, reached their highest concentrations just below the stratum corneum. The ceramides increased their concentration sharply in the granular layer; cholesterol, fatty acids and triglycerides reached a peak in the stratum corneum. These results indicate that phospholipids are degraded during terminal differentiation of keratinocytes, acylglucosylceramide may be involved in formation of the permeability barrier at the granular/keratin junction, and ceramides and/or triglycerides may constitute the permeability barrier in the stratum corneum.

A preliminary estimate of the energy requirement for steroid diffusion and binding within human stratum corneum

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Studies of corticosteroid-induced human vasoconstriction have shown that a steroid reservoir exists in the stratum corneum which can be diminished by increasing hydration (Vickers, *Arch. Dermatol.* 1963, **88,** 20). Further confirmation has here been obtained from *in vitro* diffusion experiments on four representative steroids in whole human cadaver skin, which suggest that skin permeation by steroids is governed by two separate processes; steroid binding to sites which constitute a reservoir and diffusion of the residual mobile fraction.

Using methodology described elsewhere (Foreman *et al., J. Pharm. Pharmac.* 1977, **29,** 108) diffusion of four representative steroids has been studied in whole human cadaver skin. The fraction of material applied which was bound within the stratum corneum was approximately 0·6 in each case. This fraction appears to take no further part in the diffusion process. If the skin is occluded with plastic film, the bound fraction is reduced to roughly 0·15, whilst the rate of diffusion of the mobile fraction is roughly doubled.

A limited number of binding sites appear to exist within the stratum corneum to which water molecules are normally bound, but which have a greater affinity for steroids. The bound water is therefore displaced by steroids diffusing through the stratum corneum, and it has been estimated that the free energy change for this process is -60 ± 11 kJ mol^{-1} for nandrolone, whilst the activation energy for the diffusion process is 26 ± 9 kJ mol^{-1}. These data suggest that two processes are indeed involved, having different energy requirements, and that the steroid binding process involves a fairly strong chemical bond. Steroid binding within the stratum corneum must therefore be a major mechanism by which this structure exerts its barrier effect. Whether this is specific to steroids is not clear, but two non-steroids, urea and dimethylsulphoxide, which have a strong affinity for water, are apparently not bound within the stratum corneum in this way.

Elevated phosphodiesterase activity in atopic dermatitis monocytes: a histamine-induced effect

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Previously we have demonstrated elevated cAMP phosphodiesterase activity (PDE) in mixed mononuclear leukocytes (MNL) from atopic dermatitis (AD) patients. This study defines the cell type responsible for the elevated PDE and demonstrates a possible causative mechanism through histamine desensitization (HD).

We purified Hypaque–Ficoll isolated MNL by differential centrifugation on Percoll gradients to yield 64% monocyte rich (MR) and 94% lymphocyte rich (LR) fractions. Adherence techniques yielded monocytes of 94% purity (AM) and non-adhered cells (NC) containing 85% lymphocytes. PDE activity was quantitated by radioenzyme assay of cell homogenates expressing data as pmol cAMP/min/10^6 cells. The effect of HD on PDE was investigated by incubating normal MNL with 10^{-6} M histamine before separation into subsets.

MNL, MR and AM showed significantly elevated PDE activity in AD and histamine-treated MNLs when compared with normal controls ($P < 0{\cdot}005$).

TABLE

	Normal (*n*)	AD	HD
MNL	0·026+0·005 (9)	0·069+0·006 (9)	0·119+0·007 (6)
MR	0·019+0·005 (8)	0·052+0·008 (9)	0·354+0·04 (6)
AM	0·042+0·008 (6)	0·207+0·05 (4)	0·242+0·04 (5)

Less activity was noted in the lymphocyte rich preparations and the difference between AD, HD and normals was far less marked. The increase in PDE activity caused by histamine was partially blocked by cimetidine in the MNL and LR but completely blocked in the MR.

Numerous abnormalities of monocyte function are found in AD. Loss of cyclic nucleotide regulation of monocyte function secondary to histamine stimulation of PDE activity may cause these abnormalities.

Adrenomedullary function in atopic eczema

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Impaired mononuclear leukocyte (MNL) cAMP responses in atopic eczema (AE) have been suggested to be secondary to desensitization following exposure to chronically elevated levels of catecholamines (CAs) (and other compounds). The aim of this study was to determine whether the adrenal medulla in patients with AE responds abnormally to known stimuli of CA secretion.

Plasma adrenaline and noradrenaline levels were estimated in atopic and control subjects ($n = 6$/group) after (i) lying supine for 20 min, (ii) standing for 10 min, (iii) a 5 min i.v. infusion of histamine (0·1 μg/kg/min base) and (iv) a 15 min i.v. infusion of adrenaline (0·025 μg/kg/min). In addition, plasma cAMP levels, heart rate and blood pressure were monitored.

There was no significant difference between resting plasma CA levels in the atopic and control groups. Plasma CA levels in response to histamine infusion and standing were elevated in both groups but there were no significant differences between atopic and control subjects. Infusion of exogenous adrenaline resulted in increased plasma levels of both adrenaline and cAMP; these values did not differ significantly between the atopic and control groups.

This *in vivo* study indicates that, for a given stimulus, adrenal medullary responses in AE are normal. Patients with AE also metabolize adrenaline at a normal rate. Thus, abnormal *in vitro* MNL cAMP responses in AE do not appear to be associated with abnormal adrenomedullary function.

Intestinal permeability in adults with atopic eczema

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It has been suggested that an abnormal intestinal reaction to dietary reagins increases small bowel permeability and may be relevant to the pathogenesis of atopic eczema (Atherton, *Clin. Exp. Dermatol.* 1983, **8**, 227). This study investigated the small intestinal permeability of patients with atopic eczema by measuring the percentage urinary recovery of cellobiose and mannitol: an established and sensitive index of passive permeability in the small bowel (Cobden *et al.*, *Gut* 1980, **21**, 512).

Twenty-nine out-patients with mild eczema and thirty patients hospitalized by severe eczema were studied and the results compared with those of fifty control subjects known to possess normal jejunal mucosa. Each subject ingested a hypertonic solution containing 2 g mannitol and 5 g cellobiose, after an overnight fast, and their urine was collected for 5 h. The percentage urinary recovery of cellobiose and mannitol was measured by paper chromatography and spectrophotometry and the cellobiose/mannitol recovery ratio calculated.

The results shown in the table indicate that there was no difference in the urinary recovery of cellobiose and mannitol between the patients with eczema and normal controls.

	Cellobiose	Mannitol
Controls	0·32% (±0·29%)	20% (±8%)
Mild eczema	0·3% (±0·4%)	24% (±16%)
Severe eczema	0·25% (±0·14%)	23% (±13%)

The cellobiose/mannitol recovery ratio was abnormally raised in four patients with mild eczema and in three with severe eczema. The jejunal histology was normal in five of these patients and one had occult coeliac disease.

This study suggests that the small bowel permeability is normal in the majority of patients with atopic eczema.

The influence of serum and plasma from patients with systemic sclerosis on behaviour of normal erythrocytes and function of normal neutrophil leukocytes

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There are abnormalities of the serum and plasma in patients with systemic sclerosis (SS) and there are reports of these patients' sera altering the *in vitro* behaviour of normal lymphocytes, fibroblasts and endothelial cells.

We previously have reported decreased relative filterability of red blood cells (RBC) from patients with SS (Kovacs *et al.*, *Clin. Sci.* 1983, **65**, 515). Using the same filtration technique, we incubated homologous normal fresh or frozen plasma, or frozen serum with washed control RBC and found no change in the filterability. However incubation of washed control RBC with fresh or frozen plasma, or frozen serum from SS patients significantly reduced their filterability ($P<0{\cdot}01$).

There was a 3·5-fold increase in the adherence of normal RBC to cultured human endothelial cells when plasma from SS patients rather than control subjects was added to the incubation medium ($P<0{\cdot}001$). To ensure that the increased adherence was not due to the cytotoxicity of SS plasma during the 1 hour incubation, the uptake of adenine by endothelial cells was measured in a separate study. There was no difference in the uptake when either control plasma or SS plasma was added to the medium.

Whole blood chemiluminescence of neutrophils from normal subjects was induced by adding opsonised zymosan particles. Frozen serum from patients with SS significantly increased the chemiluminescence compared with the effect of control sera, $P<0{\cdot}001$.

This study has shown that serum and plasma from patients with SS alter the *in vitro* behaviour of normal RBC and neutrophil leukocytes. Further investigation and isolation of the factors responsible for these changes may be helpful in understanding the pathogenesis of systemic sclerosis.

Quantification of nail fold capillary abnormalities in Raynaud's syndrome and systemic sclerosis

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Nail fold capillary microscopy in patients with systemic sclerosis (SS) shows a characteristic pattern of enlarged capillary loops with surrounding avascular areas in 95% of cases. Similar changes can be seen in dermatomyositis and mixed connective tissue disease and occasionally in primary Raynaud's disease without evidence of underlying disease (RD) or in Raynaud's phenomenon occurring in other connective tissue diseases (RP). A subjective quantification of capillary abnormalities was shown to correlate with disease severity in SS (Maricq *et al.*, *Am. J. Med.* 1976, **61**, 862), a finding disputed by Lee *et al.* (*J. Rheum.* 1983, **10**, 930). The present study was designed to quantify objectively the capillary changes in terms of capillary numbers and luminal diameters.

Four groups were studied: nineteen normal controls, ten RD, twelve RP and eighteen SS. Under standardized conditions, capillaries were photographed via a binocular microscope. Capillary numbers were counted per linear mm and per 0·5 mm^2 by projection at a final magnification of ×260; the luminal diameters of the afferent and efferent capillary loops were measured by the same technique at ×1000 magnification.

The results show reduced mean capillary numbers per 0·5 mm^2 in all three groups, compared with the controls: normal 13·8, RD 12·4 ($P<0{\cdot}05$), RP 10·1 ($P<0{\cdot}001$), SS 6·9 ($P<0{\cdot}001$). The mean luminal diameters for the efferent and afferent loops respectively were: normal 10·4 μm and 8·0 μm, compared with 19·6 μm and 15·0 μm for the SS group ($P<0{\cdot}001$). Results will be presented for the luminal diameters in RP and RD, together with a correlation with disease severity and duration in SS.

Our results show a reduction in capillary numbers in RP/RD which has not previously been quantified, and suggest that there is a continuous spectrum of capillary changes from normal to SS rather than a pathognomonic change in SS. Long term follow up will be needed to confirm this impression.

MSH and the control of coat darkening in the Agouti mouse

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The Agouti mouse (C3H/He A^{*vy}) undergoes changes in coat colour and at puberty grows a darkly pigmented coat due to the presence of eumelanin. In later life this is replaced by a golden yellow coat as the synthesis of phaeomelanin predominates. Although our previous findings using bromocriptine and bromocriptine with α-MSH indicate that α-MSH is responsible for the coat darkening during puberty, we found plasma α-MSH concentrations to be similar in pubertal and adult mice. In the present study we have therefore examined the possibility that there are differences in melanocyte sensitivity to α-MSH in pubertal and adult Agouti mice. Hair was plucked from the dorsal skin of pubertal mice (dark coat) and adult mice (golden yellow coat). The mice then received daily injections of either 5 or 10 μg α-MSH/10 g body weight for up to 17 days by which time new hair had regrown in the plucked area. The darkness of this newly grown hair was measured with a reflectometer. Mice were killed at intervals during the regrowth of their hair and samples of skin taken for the measurement of tyrosinase. Tyrosinase activity increased in both pubertal and adult mice after plucking, reaching peak levels at around 8–10 days. However, the peak value in the adult mice was never greater than 50% of that found in pubertal mice. α-MSH produced no further increase in tyrosinase activity in adult mice but in the pubertal mice peak tyrosinase activity was increased by approximately 80%. Administration of α-MSH also failed to affect coat colour in the adult mice although it increased coat darkening in the pubertal mice. Thus a higher level of tyrosinase activity and an increased response to α-MSH are associated with the coat darkening of puberty than with the golden-yellow coat that occurs during adult life. It is therefore concluded that the pigmentation of the hair at puberty is related to the responsiveness of the hair follicular melanocytes to α-MSH at this time.

Enhanced responsiveness of dystrophic epidermolysis bullosa fibroblasts to interleukin-1

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It has been suggested that there is an increased activity of collagenase in cultured skin fibroblasts of patients with recessive dystrophic epidermolysis bullosa (DEB) and that this may be important in the development of blisters (Bauer *et al.*, *J. Invest. Derm.* 1983, **81**, 163). We have investigated the release of collagenase by cultured skin fibroblasts in response to interleukin-1 from two patients (one mild, one severe) with recessive DEB and from one patient with dominant DEB. The inhibition of this response by dexamethasone was also assessed. Interleukin-1 is known to enhance the production of collagenase from synoviocytes *in vitro*, and dexamethasone in physiological concentrations suppresses this effect.

Fibroblasts were grown in microwells, and collagenase activity was measured after activation with 4-aminophenylmercuric acetate. Initially, control fibroblasts degraded less than 1·5 μg collagen/h/well, and there was no detectable increase in collagenase production on incubation with interleukin-1. However, fibroblasts from the subject with severe recessive DEB responded to incubation with interleukin-1 (at a dilution of 1 in 5000) by an increase in collagenase production of 240%. Fibroblasts from the patients with localized recessive DEB and dominant DEB were stimulated to a similar extent by interleukin-1, with a resultant increase in collagenase production of approximately 100%. Dexamethasone suppressed the interleukin 1-evoked response in fibroblasts from all three cases of DEB, but the concentration required and the extent of the response were variable.

These findings suggest that there is an intrinsic abnormality of collagenase production by dermal fibroblasts in both the recessive and dominant forms of DEB.

A UVR detection system using a BBC microcomputer

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The purpose of this communication is to report on the suitability of the BBC model B microcomputer for on-line UV data storage and analysis. It provides the basis for an inexpensive, flexible system which, after 12 months continuous use, has shown an acceptable level of reliability. The new system replaces an earlier one for monitoring natural UV levels reported to the Society previously. The BBC micro has several attractive features for this particular application: a UV detector may be connected directly without external digitization and an internal clock may be accessed for a record of real time.

Sampling of data is under control of the program and is performed at 1 second intervals. At present two input channels are used. One of these is connected to the UV detector which has been calibrated to read total daylight UV irradiance. The other receives data from a visible light detector whose response matches that of the human eye. Data is usually printed out and stored on tape on a daily basis but, failing this, the computer will store up to 10 days' data within its own memory.

The microcomputer has been in continuous use 24 hours per day, 7 days a week for 12 months. In this time a total of 12 days have been lost. A mains supply failure one weekend accounted for three of those days and program development another five. The remaining lost days were due to corruption of the program while it was running.

The purpose of monitoring visible light levels as well as UV irradiance is to investigate the relation between these two parameters. It appears that UV and visible levels are closely related but that shade affects visible light more strongly than UV.

Dermatofibrosarcoma protuberans: an immunoperoxidase study utilizing antisera to lysozyme, α_1-antichymotrypsin and S-100 protein

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The purpose of this study was to determine the histogenesis of the tumour cells in dermatofibrosarcoma protuberans (DFP). DFP, a not uncommon locally aggressive cutaneous sarcoma, is thought by many to have a fibrohistiocytic origin (O'Brien & Stout, *Cancer* 1964, **17**, 1445) yet other authors have considered a neural origin to be more likely (Hashimoto *et al.*, *Arch. Dermatol.* 1974, **110**, 874).

Thirteen histologically typical tumours were treated with antisera to lysozyme, α_1-antichymotrypsin (histiocytic markers) and S-100 protein (a neuroectodermal marker). Controls included normal vagus nerve, a large intestinal abscess (diverticulitis), five benign neural tumours, two malignant nerve sheath tumours, three leiomyosarcomas, two fibrosarcomas and four pleomorphic malignant fibrous histiocytomas (MFH). All thirty-one specimens were examined with each of the antisera using a PAP technique. The reaction product was demonstrated with diaminobenzidine tetrahydrochloride.

Histiocytic markers were demonstrated in the tumour cells of MFHs and also in histiocytes present within a variety of tumours including all DFPs. The spindle cell component of the latter was uniformly negative. Antisera to S-100 protein labelled peripheral nerves, all benign neural tumours, one malignant neural tumour and occasional histiocytes (presumed Langerhans cells) but the DFPs were completely negative.

In this study the tumour cells of DFP did not label with antisera to histiocytic or neural antigens. Since positive control staining was demonstrated in MFHs and neural tumours these findings do not support an origin of the tumour cells in DFP from either histiocytes or neural cells. This suggests that DFP may be a tumour solely derived from fibroblasts.

Why are subjects with multiple contact allergies more sensitive to DNCB?

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We have previously shown that people who develop multiple (3 or more) contact allergies to environmental substances, are more susceptible to experimental sensitization with dinitrochlorobenzene (DNCB). We have now compared their dose-response relationships with normal subjects in order to establish the mechanism. In both groups, response to DNCB challenge measured as increase in skinfold thickness, is linearly related to log challenge dose. Leftward displacement of the challenge-dose response (CDR) curve, with increase in sensitizing dose can be plotted to give a log sensitizing-dose response (SDR) curve, which is also linear. In people with multiple allergies, the slope of the CDR curves was normal, but the SDR curve was much steeper than normal ($P < 0{\cdot}01$) showing that induction of sensitivity, rather than expression, is enhanced in these subjects. Possible reasons are that these people are a genetically determined subgroup with increased efficiency or number of antigen-presenting cells. Alternatively they may be a heterogeneous group at the end of the normal range. Morphology and numbers of ATPase-stained Langerhans cells (739/mm^2, s.e. = 22) were no different from normal (720/mm^2, s.e. = 60); we have not yet tested their functional competence *in vitro*. We analysed the HLA, A, B and DR phenotypes of fifty-three subjects with multiple allergies. Overall there were no associations but interestingly, there was an increased frequency of DR4 (20/29, 69%, cf 33% of 325 controls, $P < 0{\cdot}01$) in those who included nickel among their sensitivities and of DR6 (8/14, 57%, cf 16% of controls, not significant) for those sensitive to rubbers. Our evidence indicates this heterogeneous group of susceptible subjects has no common HLA phenotype, but there are specific subgroups in which antigen-specific responses may be HLA-determined.

Expression of Class II antigens in normal human skin

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It is now accepted that in order to produce a contact hypersensitivity reaction, foreign compounds must be presented to immunocompetent cells by cells bearing Class II antigens of the major histocompatibility system.

We have studied the expression of Class II antigens and their relationship to Langerhans cells (LC) in the skin of twelve normal human subjects, using monoclonal antibodies to thymocyte-like antigens (OKT6), HLA-DR framework (OKT Ia1), HLA-DR α-chain (DA6 147), HLA-DR β-chain (DA6 164), both HLA-DR β-chain and other Class II antigens (DA6 231), HLA-DC (Leu-M10) and HLA-SB (MHM4), in the indirect immunoperoxidase reaction.

In the epidermis, regularly arranged dendritic cells were seen in the same distribution with all antibodies except MHM4. Basal keratinocytes were positive with DA6-164 and Leu-M10, and the majority of keratinocytes were positive with DA6-231 and MHM4. Cells lining the intraepidermal sweat ducts were

HLA-DR, HLA-DC and HLA-SB-positive. In the dermis, OKT6-positive cells were sparse, but large numbers of positive cells were seen with all other antibodies, mainly round appendages and blood vessels. Endothelial cells were positive with all antibodies to Class II antigens.

These findings are consistent with the view of the LC as an antigen-presenting cell, and the evidence from other tissues that HLA-DR and HLA-DC may both be necessary for this function in macrophages. That keratinocytes also bear Class II antigens might support the theory that these cells are capable of presenting antigen. The appendages, particularly the sweat ducts, may also be important in the handling of applied antigens, as their cells bear HLA-DR, -DC and -SB and they are the focus of activity of antigen-presenting cells in the dermis.

Differentiation markers in lymphoid infiltrates

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An increasing number of monoclonal antibodies available to maturation, activation and proliferation markers on T cells are being used to further our understanding of the mechanism of cutaneous T-cell malignancies as well as an aid to earlier diagnosis.

We have used a panel of monoclonal antibodies including OKT9 (anti-transferrin receptor), found on activated T lymphocytes and tumour lines, OKT10, which marks thymocytes and bone marrow precursor cells, Leu 8, which reacts with an antigen on the surface of a regulatory subset of T cells, and Ki67, an antibody to a proliferation antigen found in the nuclei of cells in active cell division.

Twenty-four biopsies comprising mycosis fungoides, suspected mycosis fungoides, and conditions included in its differential diagnosis, have been studied with these antibodies using an indirect immunoperoxidase technique. Our results so far indicate few cells staining with OKT10 in most cases, 5–20% of the infiltrate positive for OKT9 in the mycosis fungoides and lymphomas, but also cells positive for this marker in some benign conditions studied.

A smaller number of cases (15) stained with Leu 8 stained 1–10% positive cells present in 70% of biopsies regardless of histology, with the exception of one lymphoma in which the infiltrate was 80% positive for Leu 8.

The proliferation marker Ki67 was positive only in a small percentage of the infiltrate of mycosis fungoides, lymphoma or lymphomatoid papulosis cases.

No single marker has yet been demonstrated to be specific for lymphoid malignancy. Therefore future developments in this field will probably depend on the use of a panel of antibodies including these differentiation and proliferation markers.

Antigen presenting cells in atopic dermatitis—evidence for a cell-mediated immune response in the pathogenesis

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Evidence is accumulating to suggest that epicutaneous antigen plays a role in the pathogenesis of atopic dermatitis (Mitchell *et al.*, *Lancet* 1982, **i**, 27). However respiratory or gastrointestinally derived antigens may be equally important. A variety of cell types are known to act as antigen-presenting cells (APCs) including macrophages (M), interdigitating reticulum cells (IRC) and Langerhans cells (LC). Two new monoclonal antibodies (McAbs), RFD1 and RFD2 which recognize the cell surface antigens expressed by IRCs and Ms respectively, and both NA1/34 and OKT6 which identify LCs were utilized in a fast red immuno-alkaline phosphatase reaction on cryostat sections from nine atopic eczema patients, and their presence correlated with the presence of T lymphocytes and their subsets using appropriate McAbs.

A large proportion of mononuclear dermal cells were RFD1$^+$ IRCs. There were fewer RFD2$^+$ Ms with similar numbers of LCs. The majority of lymphocytes were of Leu 3a$^+$ helper phenotype. Abnormal focal dermal collections of OKT6/NA1/34 cells also exhibited the presence of surface HLA-DR antigen (OKIa$^+$) when examined by combination immunofluorescence techniques suggesting that they are true LCs rather than immature thymocytes.

LCs (and possibly IRCs) are likely to be involved in the presentation of epicutaneous antigen to T lymphocytes resulting in chronic contact hypersensitivity. Conversely the microenvironmental relationship between abundant RFD1$^+$ cells and Leu 3a$^+$ lymphocytes may indicate that IRCs are presenting internally derived antigen to helper T lymphocytes in a local immunological response. Such mechanisms may be responsible for the more persistent inflammatory reaction which characterizes chronic atopic eczema lesions.

Anthralin increases lipid peroxide formation in skin and free radical scavengers reduce anthralin irritancy

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A limitation to anthralin in the treatment of psoriasis is the inflammation which often limits the dose that can be given. Anthralin forms free radicals in solution and in the present work we have investigated the possibility that these free radicals cause lipid peroxidation and irritancy in two separate studies.

(1) When added to mouse skin microsomal fractions, anthralin stimulated (+50%) the rate of formation of lipid peroxides as measured by malondialdehyde production. The rate of peroxidation in the presence of anthralin was increased by the addition of NADPH (50%), and inhibited by tocopherol (80%), butylated hydroxyanisole BHA (40%), carotene (60%) and EDTA (40%), indicating that reactive oxygen metabolites were responsible for peroxide formation. Mannitol, dimethylurea and sodium benzoate did not inhibit lipid peroxidation induced by anthralin indicating that hydroxyl radicals were not active in peroxidation.

(2) In clinical studies the free radical quenching agents tocopherol, EDTA and BHA, when applied simultaneously with anthralin under occlusion, were found to inhibit anthralin irritancy as measured by the increase in skinfold thickness and erythema. These results suggest that anthralin irritancy may be related to free radical generation and lipid peroxidation.

Determination of elastin in normal dermis during ageing

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Techniques for the biochemical determination of elastin in tissues depend on destruction of other tissue components, including collagen and glycoproteins. Methods such as autoclaving or prolonged extraction with sodium hydroxide are effective in the purification of elastin from aorta or ligamentum nuchae, which are rich in elastin. These destructive techniques are unsatisfactory when applied to dermis which contains only 3–5% elastin.

We have compared several methods of purification of elastin from human skin. These include treatment with collagenase, urea, guanidinium chloride, cyanogen bromide, β-mercaptoethanol, sodium hydroxide or a combination of methods. The purity of the residue has been determined using amino acid analysis.

The most satisfactory technique proved to be limited digestion of skin with 0·1 M sodium hydroxide in a boiling water bath for 35 min. Using this method, we have examined samples of abdominal skin from normal humans covering a wide age range of 0·2 to 85 years. There was no change in the weight of residue (approx. 5% of the total starting weight of freeze-dried material). Furthermore, no age-related changes were demonstrated in the amino acid composition of the material. This is in contrast to our findings in skin subjected to chronic sun damage in which we detected a substantial increase in elastin. We conclude that ageing of skin, in the absence of sun damage, does not lead to alteration in elastin.

Premalignant and malignant lesions of the epidermis retain their essential characteristics when grafted to athymic mice

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Solar keratoses may progress to frank neoplasia, regress or remain unchanged for long periods. The determinants of their behaviour are unknown and insight into them would help in understanding the biological significance of preneoplasia. Our aim has been to characterize the fate of solar keratoses and squamous cell carcinoma in an immunologically privileged environment by transplanting such lesions to athymic mice (Nu/Nu). A total of twenty-five lesions have been grafted, of which to date 75% were successfully retained for periods of up to 6 months. Grafted lesions have been examined histologically and compared morphometrically with the original lesions. Grafted lesions retained most of the morphological features of the original lesions although in some cases the epidermal thickness was reduced and cell size was slightly reduced. In addition lesions were examined autoradiographically after incubation in the presence of tritiated thymidine in order to characterize the rates of epidermal cell production. The mean labelling index was 11·2% for nine lesions measured, compared to 6·1% for normal skin, suggesting that there was no change in the enhanced proliferation usually encountered in these lesions. The human origin of the grafted tissue was confirmed using an antibody to involucrin as this precursor protein is specific to human epidermis.

The study demonstrates the utility of the model, and results thus far suggest that solar keratoses do not appear to be 'held in check' by cell-mediated immunity *in vivo* in man and may be independent lesions *sui generis*.

The bacteriology and antibiotic treatment of suppurative hidradenitis

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Bacteriological study of lesional discharge from thirty-six patients with chronic suppurative hidradenitis identified a plausible pathogen at least once in twenty-six of thirty-two patients with perineal disease, eleven of seventeen with axillary disease, two of three with breast disease, and one of two with neck lesions.

Group F streptococci (*Streptococcus milleri*) and anaerobic streptococci were isolated from all these sites; *Staphylococcus aureus* from axillae, perineum and breasts; and *Bacteroides spp.* from perineum only.

Statistical analysis was performed on the data from the largest group (perineal). In these patients the main pathogen was the Group F streptococcus whose presence was significantly associated with disease activity and whose disappearance significantly correlated with clinical improvement. Significant improvement was associated also with the disappearance of *Staph. aureus* and of anaerobic streptococci.

Bacteriologically appropriate treatment afforded substantial improvement in over a quarter of our cases. The persistence of the underlying anatomical distortion is probably the main reason for failure or limited response in the others, but the ineffectiveness of penicillins in this disease against Group F streptococci, and the ready development in that organism of resistance to erythromycin and tetracyclines, also complicate management.

X-ray microanalytical studies of Na, K and Cl levels in resting and active human sweat glands

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Sweat glands at different stages of activity were examined by X-ray microanalysis as a step towards elucidation of the electrolyte transport mechanisms. Skin samples, 4 mm diameter, were obtained by biopsy without anaesthesia, using a high speed punch technique, from the backs of four adult males. Samples were obtained before and after thermally induced sweating which was continuously monitored on the contralateral side using ventilated capsules and a humidity measuring system (Humitec). Ambient conditions were 18°C, 25% RH at initial sampling and 43°C, 35% RH during sweating.

The tissue was subdivided and cryo-quenched by rapid entry into propane at −192°C (Elder *et al., J. Microsc.* 1982, **126**, 45). After storage in liquid N_2, samples were freeze-dried at −85°C for 3 days, followed by a slow 6 h warm up to 0°C and vacuum embedding in Araldite. Sections of secretory coils and ducts were dry-cut at 150 nm on a diamond knife, carbon coated and analysed at −160°C on a cold stage (Nicholson *et al., J. Microsc.* 1982, **126**, 307) in a modified JEOL 100C TEM (Nicholson *et al., J. Microsc.* 1982, **125**, 25).

Thirty elemental spectra were taken from the cytoplasm of the secretory epithelium in each instance and dry weight mass fractions were computed. Results on the fundus indicated a dramatic fall in potassium concentration during sweating ($P < 0.01$). In contrast the sodium and chloride concentrations were elevated ($P < 0.05$). These data provide a basis for continuing physiological and pathological studies.

A study of the membranous ATPase system of Langerhans cells after epicutaneous application of a sensitizer (DNFB) or a tolerogen (DNTB)

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We have carried out a comparative study of the fate of Langerhans cells (LC) after epicutaneous application of two haptens: 2,4-dinitrofluorobenzene (DNFB) and 2,4-dinitrothiocyanobenzene (DNTB), the former being a powerful sensitizer and the latter a tolerogen, in epicutaneous application.

For this study, samples were taken in the square used for induction of sensitivity or tolerance at 24 h intervals, during the 5 or 7 days, respectively, necessary for the development of allergy or tolerance to DNFB (DNFB indeed crossreacts with DNTB). In order to visualize and to count LC, we estimated their membranous ATPase activity.

The results of LC enumeration are shown in the Table.

TABLE

	Variation in LC density (LC/mm²) during induction of:	
Day	Sensibility to DNFB	Tolerance to DNFB by DNTB
0	893 ± 46	893 ± 46
1	273 ± 87	895 ± 46
2	582 ± 67	788 ± 49
3	—	864 ± 9
4	902 ± 100	776 ± 8
5	919 ± 94	875 ± 45

Whereas DNFB caused a rapid and important although transitory fall in the density of ATPase positive LC, DNTB did not significantly modify this density.

These findings stress the vital role of membrane ATPase in the function of LC which seem to constitute the critical element for determining whether exposure to a hapten induces a state of sensitivity or tolerance.

IgG$_4$ antibodies against the dermo-epidermal junction in bullous pemphigoid

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In bullous pemphigoid (BP) the formation of blisters is thought to be a consequence of activation of complement and granulocytes by antibodies (Ab) against the dermo-epidermal junction (DEJ). However, non-complement fixing Ab of IgG$_4$ subclass was found by Sams & Schur (*J. Lab. Clin. Med.* 1973, **82**, 249) and in other autoimmune disorders such as Graves' disease, IgG$_4$ auto-Abs are also found. We have studied the subclasses of anti-DEJ Ab with specific monoclonal antibodies and examined the possibility that there is a general defect of regulation of IgG$_4$. Direct immunofluorescence (IF) examination of perilesional skin from 18 BP patients showed linear IgG$_4$ in seventeen, IgG$_1$ in twelve, IgG$_3$ in five, C3 in sixteen (both C3 negative biopsies contained IgG$_4$ only). Of twenty-three BP sera tested for DEJ-Ab by indirect F, it was absent in six, of IgG$_4$ subclass in seventeen, and of both IgG$_4$ and IgG$_1$ subclasses in fourteen. The titre of IgG$_4$ was > IgG$_1$ in 16/17. The anti-DEJ IgG$_4$ contained both κ and λ light chains in 6/6 tested, indicating heterogeneity. Thus in BP, non-complement fixing IgG$_4$ is the predominant and sometimes the only Ab subclass against the DEJ, which throws doubt on the role of complement. IgG$_4$ is a minor Ig subclass in serum, about 5% of total IgG. The serum levels of IgG$_4$ (assayed by ELISA) in twenty-three BP patients did not differ from those in age-matched controls. Specific IgG$_4$ Abs to milk and egg (IgG$_4$ RAST), gliadin and respiratory syncytial virus (ELISA) were present but their titres did not differ from those in controls. Thus there is not a generalized increase in IgG$_4$. Total IgE was increased but no Ab specificities against foods or inhalants were detectable with RAST tests. In addition, no IgE Ab against the DEJ was detected by IF or autoradiography. The contribution of IgG$_4$ anti-DEJ Abs to pathogenetic mechanisms remains to be determined.

Expression of HLA-DR in the hair follicle in alopecia areata

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The expression of HLA-DR by cells normally HLA-DR$^-$ has been documented in a number of disease states in which immune mechanisms are implicated. The follicular lesion in alopecia areata (AA) is widely believed to be mediated immunologically but the evidence for this is largely circumstantial. In this study the expression of HLA-DR in the hair follicle in AA was investigated by immunohistochemistry of tissue sections using a monoclonal anti-HLA-DR antibody. Scalp biopsies were taken from eight patients with AA and from five normal individuals. In normal follicles HLA-DR expression was confined to dendritic cells (presumably Langerhans cells) which were sparse below the level of arrector insertion. Five of the eight AA biopsies were taken from bald patches or from areas showing regrowth of non-pigmented hair. Some or all of the anagen follicles in these five biopsies showed HLA-DR expression on keratinocytes of the presumptive cortex and in the dermal papilla. In one biopsy, outer root sheath cells were also HLA-DR$^+$.

The perifollicular lymphoid infiltrate was also HLA-DR$^+$ but the intensity of the infiltrate around follicles expressing HLA-DR was very variable. In the other three AA biopsies which were taken from uninvolved areas and from areas showing regrowth of pigmented hair HLA-DR expression in the hair follicle was seen only on dendritic cells.

Previous morphological studies have shown that cell injury in the hair follicle in AA is confined to the presumptive cortex. The demonstration that cells of the presumptive cortex preferentially express HLA-DR gives added support for the hypothesis that the pathogenesis of the disease involves this differentiating compartment of the anagen follicle. The biological role of aberrant HLA-DR expression is uncertain. However, these findings provide further evidence that AA is mediated by an immune mechanism. The intensity of the perifollicular infiltrate is not a reliable guide to disease activity.

HTLV1 in cutaneous T-cell lymphoma—a serological study in British patients

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HTLV1 is an oncogenic retrovirus that has been found to cause a particularly malignant form of cutaneous T-cell lymphoma (CTCL) which is endemic in Haiti and in isolated regions of Japan. In this study we have examined patients with CTCL attending St Johns Hospital for serological evidence for the presence of this virus. Fifty-six patients with CTCL, skin stages T1–13, T2-12, T3-13 and T4-19, were examined. Ten millilitres of clotted blood were taken from each patient and the serum was separated. Target cells, a human T-cell line transformed with and producing HTLV1 virus (provided by Prof. R. Gallo) were grown in tissue culture and harvested when in exponential growth. The cells were washed in PBS, and then incubated first with the patients' serum neat or diluted 1 in 4 in PBS, washed three times in PBS and then incubated with a fluorescein-conjugated rabbit anti-human immunoglobulin. The cells were then fixed in 3% paraformaldehyde, washed in PBS and mounted in buffered glycerine and examined on a Zeiss fluorescence microscope. As a negative control, serum from ten healthy volunteers was used, and as a positive control, serum from a patient shown by Prof. Gallo's lab to have anti-HTLV1 antibodies was used. The positive control gave 100% bright fluorescence of the cells. All the controls and all the patients' serum tested were consistently negative.

This study suggests that HTLV1 is not the aetiological agent in the form of CTCL seen in Europe. It is however possible that a related virus which is not serologically cross-reactive with HTLV1 is responsible.

Evidence that the raised level of phospholipase A_2 in the uninvolved epidermis of psoriasis is caused by hyperphosphorylation of an inhibitor

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Phospholipase A_2 activity from non-lesional psoriatic epidermis is significantly greater than that from normal epidermis (Forster *et al.*, *Br. J. Derm.* 1983, **109**, Suppl. **25**, 30). We have now shown that when an extract from uninvolved psoriatic epidermis is mixed with an extract from normal epidermis, the mean phospholipase A_2 activity in the mixture is only 58% of the sum of the activities in the components ($n = 7$). This suggests that normal epidermis contains an inhibitor of phospholipase A_2.

We have also incubated extracts of uninvolved psoriatic epidermis with insolubilized alkaline phosphatase before measuring phospholipase A_2 activity: such treatment reduced the enzyme activity into the normal range. This observation is consistent with the presence in epidermis of an inhibitor whose inhibitory activity is inversely proportional to its degree of phosphorylation, as has been demonstrated in rat macrophages (Hirata *et al.*, *Biochem. Biophys. Res. Comm.* 1982, **109**, 223).

If epidermal phospholipase A_2 activity is controlled by a phosphorylated protein, and if epidermis contains a protein phosphatase capable of removing phosphate groups from an inhibitory protein, one might expect that inclusion of the protein phosphatase inhibitors fluoride or pyrophosphate (Resink *et al.*, *Eur. J. Biochem.* 1983, **133**, 455) in the homogenizing medium would increase phospholipase A_2 activity compared with homogenates prepared in the absence of inhibitors. We have shown that this is indeed the case.

From these observations, we suggest that epidermis contains a phosphorylated inhibitor of phospholipase A_2 and that in non-lesional psoriatic epidermis, the inhibitor is hyperphosphorylated leading to the observed increase in phospholipase A_2 activity seen in this tissue.

Exacerbation of dermatitis herpetiformis induced by indomethacin

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We have found in our Dermatitis Herpetiformis clinic that a number of patients experienced an exacerbation of their skin lesions when taking non-steroidal anti-inflammatory drugs. To determine if this observation was coincidental or an effect of these drugs we carried out a double-blind cross-over study of indomethacin versus placebo in patients with dermatitis herpetiformis.

Thirteen patients whose rash was controlled with dapsone (9) or sulphamethoxypyridazine (4) alone were studied. The lowest possible dose to control the eruption was determined prior to the study. Patients were then given 25 mg t.d.s. of indomethacin or placebo for a 4-week period and then crossed over.

It was found that the skin lesions were exacerbated in nine of the thirteen patients taking indomethacin but in only one taking the placebo, necessitating an increase in dapsone/sulphamethoxypyridazine to control the rash.

Indomethacin is a known potent inhibitor of the cyclo-oxygenase pathway by which arachidonic acid is metabolized to prostaglandins. However, in this situation there may well be increased breakdown of arachidonic acid via the 5- and 12-lipoxygenase pathways leading to increased production of leukotriene B_4, 12-HETE and other inflammatory mediators. These substances could well play a part in the final pathogenic pathways of dermatitis herpetiformis.

A new strategy for identification of antigens (exemplified by β_2-microglobulin) in paraffin-embedded cutaneous tissue

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Recognition of antigens in tissue sections is sometimes hampered by low sensitivity due to small amounts of antibody combining to a limited number of epitopes on antigenetic material and to high background staining related to the binding of mammalian antisera to Fc-receptors on human tissue.

β_2-microglobulin (β_2-m) occurs on the cell surface of all human nucleated cells as part of the HLA molecule. In certain skin tumours loss of β_2-m has been associated with malignant change, indicating a possible diagnostic value for this marker as well as relevance to a mechanism of possible impaired immunological responses to cutaneous tumours. At present the most satisfactory method of identifying β_2-m in paraffin-embedded tissue is by means of a triple-layer PAP technique using heterologous mammalian antisera.

A polyclonal antiserum against human β_2-m has been raised in chickens. Because of the phylogenetic differences between the species, the resulting antiserum is likely to recognize more epitopes on the antigenic material and show greater sensitivity than antisera raised in mammalian species. To confirm this hypothesis *in vitro*, the avian antiserum was compared to both mammalian polyclonal (rabbit) and monoclonal (mouse) antibody. β_2-m, fixed to plastic surfaces, combined with more avian than mammalian antibody. Furthermore, captured chicken antibody could bind more secondary antibody–HRP conjugate than could captured mammalian antibody, thus showing even greater enhancement with this method.

Immunohistochemical analysis of these systems confirmed that the avian strategy has greater sensitivity, in an indirect technique, with considerable reduction in non-specific background activity. It appears to be the most suitable technique for identifying β_2-m in paraffin-embedded skin tissue and the principle should be valuable in the detection of other tissue antigens.

Skin oxygen tension in venous disorders of the lower limb

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Recently, it has been suggested that pericapillary fibrin deposited as the result of venous hypertension may block the diffusion of oxygen to the overlying epidermis producing cellular death and venous ulceration (Browse & Burnand, *Lancet* 1982, **2**, 243).

We have used an oxygen electrode (Hellige Oxymonitor) to measure the transcutaneous PO_2 ($TcPO_2$) on the lower leg and forearm. We have studied twelve patients with a history of venous ulceration and thirteen control subjects. Measurements were made with subjects recumbent, standing and undertaking active movement of the ankle joint.

In the recumbent position the $TcPO_2$ of the affected legs was greater than that of control legs. This finding is inconsistent with the presence of a block to the diffusion of oxygen to the epidermis in affected legs. On standing almost all subjects of both groups showed a marked fall in $TcPO_2$. Exercise in the standing position resulted in a marked rise of the skin PO_2 in the control group whereas in legs with venous ulceration a rise was observed in only one case. The diminished fall in foot venous pressure in response to exercise which has been demonstrated in affected limbs (Burnand *et al.*, *Br. J. Surg.* 1981, **68**, 297), supports our findings. The high $TcPO_2$ values recorded from affected limbs in the recumbent position explains the favourable effect of bed rest on venous ulceration.

It is clear that our study of skin oxygen tension on limbs undergoing exercise has significantly advanced the understanding of the mechanisms involved in the development of venous ulceration.

POSTERS

Lymphocyte calmodulin levels in psoriasis

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The calcium binding protein calmodulin (CaM) has recently been shown to be increased in psoriasis in both lesional (Van de Kerkhof & Van Erp, *Br. J. Derm.* 1983, **108**, 217) and non-lesional (Tucker *et al.*, *J. Invest. Dermatol.* 1984, **82**, 298) psoriatic epidermis, and CaM levels have been observed to fall after treatment. CaM is an intracellular regulator of several processes which have been shown to be abnormal in psoriatic epidermis, activating enzymes such as adenylate cyclase and phospholipase A_2.

In order to determine whether increased CaM activity is a generalized tissue abnormality in psoriasis, we have measured the levels present in a non-epithelial static population of cells, peripheral blood lymphocytes (PBLs). Venous blood samples were obtained from eleven patients with stable plaque psoriasis, and from eight non-psoriatic controls. The samples were heparinized and red blood cells, platelets, polymorphonuclear leukocytes and phagocytic mononuclear cells were removed by sequential addition of carbonyl iron granules, Dextran 150 and density gradient centrifugation. PBLs obtained were stored in Tris buffer containing protease inhibitors at −20°C until assayed. Samples were homogenized, their protein content determined, and the CaM content measured using a CaM-sensitive phosphodiesterase assay.

There was no significant difference between the levels of biologically active CaM obtained for psoriatic PBLs: $0{\cdot}69 \pm 0{\cdot}18$ μg CaM mg^{-1} cell protein ($\bar{x} \pm$ s.e.m., $n = 11$) and control samples: $0{\cdot}66 \pm 0{\cdot}16$ μg CaM mg^{-1} ($n = 8$). The levels for both groups of samples are consistent with those obtained in other human tissues, where there is between 0·5 and 1·0 μg CaM mg^{-1} cell protein on average.

These results suggest that elevated CaM activity in psoriasis may be a specific defect of the epidermis. Such a localized defect provides additional encouragement for the development of the many potent CaM inhibitor drugs, as a new class of topical treatments for psoriasis.

Effect of calmodulin and anti-calmodulin on human melanoma cell lines

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We have examined the effect of calmodulin and anti-calmodulin on the human melanoma cell line B0008. A cell suspension of B0008 was used to inoculate dishes containing various concentrations of calmodulin or anti-calmodulin in a defined serum-free medium being developed in this laboratory specifically for the culture of human melanoma cell lines. The test cultures were incubated for 5–6 days, harvested, and total cell counts made. Anti-calmodulin was tested at concentrations of 10^{-4} to 10^{-7} M and a 50% or greater reduction in cell number over the control was seen at all tested concentrations. Calmodulin was tested at concentrations of 0·001–1 unit/ml and the cell number was increased, over the control, by at least 20% at all concentrations tested. This work was repeated using serum supplemented medium. Again anti-calmodulin exhibited an inhibitory effect but the cell number was reduced by only 50% at 10^{-4} M anti-calmodulin. Calmodulin stimulated cell proliferation in the serum containing medium but the increase over the control was only 10% at the optimum concentration (0·1 unit/ml).

The results suggest that both calmodulin and anti-calmodulin can affect the proliferation of the human melanoma cell line B0008 but that the effect can be modified by the growth of the cells in serum supplemented medium. This may be explained by the fact that calmodulin-like activity has been found in fetal calf serum (Walker *et al.*, abstract 1984, *M.R.S.*). It may be that this activity counteracts the inhibitory effect of anti-calmodulin and thus prevents significant inhibition at lower concentrations of anti-calmodulin. Preliminary work has been started on other human cell lines.

Ultrastructural morphometry of mycosis fungoides: the cell contour index as a potential diagnostic aid

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In recent years morphometric techniques have been applied in the investigation of dermatological disorders. A number of reports have shown that a relatively simple indicator of neoplastic transformations of T lymphocytes, as occurs in mycosis fungoides (MF)—the nuclear contour index—is a diagnositic aid. However, other morphometric parameters may prove to be useful. In this report we have evaluated the cell contour index (CCI) of infiltrating cells in MF and in several benign dermatoses. The electron micrographs of ultra-thin sections of biopsies from normal (N) and from lesions of lichen planus (LP), pityriasis lichenoides (PL) and from patients with MF stages I-V were studied using morphometric techniques. A statistical sampling regime was used to obtain electron micrographs of whole cells with hyperconvoluted nuclei in the dermal infiltrate. These were analysed with a semi-automatic digitizing system. Perimeter trace lengths of the cells (P) and the areas (A) were determined and the CCI calculated: $CCI = P/\sqrt{A}$. The following mean results were obtained: N 3·6; LP 4·2; PL 3·7; MF Stage I 4·09; MF Stage II 4·1; MF Stage III 4·1; MF Stage IV 4·7; MF Stage V 4·8. Statistically significant differences were found in the pooled MF data as compared with those of the 'controls'.

This preliminary report indicates that the CCI is of use in differentiating MF from other dermatoses in which there is a lymphoid infiltrate.

Lectin binding to psoriatic epidermis: further evidence for altered rather than incomplete differentiation

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Biochemical studies on psoriatic keratinocytes in organ culture or suspension have shown abnormalities at all stages of glycoconjugate metabolism. It has been suggested that such changes underly the profound modifications in cell surface properties and cell–cell interactions found in the disease. To complement the biochemical data, avoid the use of proteolytic enzymes, and allow direct correlation of glycoconjugate composition with stage of differentiation *in situ*, we have assessed the binding pattern of ten rhodamine or fluorescein-labelled lectins to cryostat sections of involved and uninvolved psoriatic skin.

Comparison with the results of our previous studies on normal skin (*Br. J. Dermatol.* 1984, **111**, 517) shows that changes in lectin binding sites are found at all levels of differentiation in involved psoriatic epidermis, and also in dermal components such as capillaries and elastic fibre. However, in contrast to other conditions (e.g. basal cell carcinoma) in which the balance between differentiation and proliferation is disturbed, there is no indication that the basal-like (in terms of lectin binding sites) cell compartment is expanded. On the contrary, some lectins maintain the normal basal-suprabasal boundary, others bind closer to the basal lamina, and expression of certain normal suprabasal characteristics is even found in the psoriatic basal layer.

These findings indicate that, with respect to lectin binding sites, the observed changes in psoriatic epidermis are not secondary to hyperproliferation, and that psoriatic keratinocytes are executing an altered programme of differentiation rather than one which is incomplete due to reduced transit times.

Quantitative studies on ultraviolet erythema

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A reflectance instrument has been used to quantify ultraviolet erythema in human skin and obtain a dose-response relationship. Five normal subjects and three patients with chronic actinic dermatitis were studied. The degree of erythema was measured at adjacent sites on the mid-back before exposure to incremental doses of ultraviolet radiation obtained from an unfiltered xenon arc lamp. The increase in erythema at 24 h compared with pre-irradiation measurements was plotted against the logarithm of the erythemally effective dose of ultraviolet radiation.

In each case the initial part of the response was non-linear but from the approximate region of the minimal dose to result in visible erythema, a linear relationship was found. Although doses of ultraviolet radiation were given that resulted in painful burns, a flattening off of the response was not seen in normal subjects. There was no significant difference in slope for the normal subjects and two of the photosensitive patients, in whom a parallel shift of the response to the left was observed. In the remaining subject with chronic actinic dermatitis, a reduced slope was obtained with a flattening off of the response at a relatively mild degree of erythema.

Dose-response data should yield more information concerning the mechanism of ultraviolet erythema than the minimal erythema dose, a detection threshold measurement.

Are sunscreen protection factors misleading?

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The measured value of the sun protection factor (SPF) for any given sunscreen depends on the methodology used. We have standardized our method as carefully as possible in order to measure and compare the SPFs of ten different proprietary sunscreen preparations and assess their reproducibility in each case.

A xenon arc solar simulator light source (Kratos 2500W) was used since its spectral output closely resembles that of terrestrial sunlight. Each sunscreen was tested on at least ten subjects and in accordance with the U.S. Food and Drugs Administration guidelines (Federal Register, Department of Health, Education and Welfare, U.S.A., vol. 43, 1978). Each sunscreen was applied in a concentration of 2 $\mu l/cm^2$ to a 2 cm^2 area of skin on the subject's back. Responses were read at 24 h by two observers, with the aid of a white fluorescent lamp. The SPF was calculated as the ratio of the time required to produce minimal erythema on sunscreen protected skin to the time required to produce minimal erythema on unprotected skin.

Dosimetry showed a 2·5-fold variation in beam intensity which was corrected by application of a correction factor for each test site. The coefficient of variance of the SPF ranged from 14% to 57% with an average value of 30·4% for the ten tested sunscreens. Representative mean SPFs obtained and their 95% confidence limits include: RoC 10, 9·4 (7·04–11·8), individual values ranged from 4 to 15·8; Uvistat, 4 (3·4–4·6), individual values ranged from 3·4 to 4·9. We feel that it is clinically extremely misleading to quote an exact SPF for sunscreens and that suncreens would be more satisfactorily classified within broad categories of effectiveness.

Production of pemphigus vulgaris-type acantholysis by a non-immunological stimulus

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Human skin is known to contain a wide spectrum of proteolytic enzymes which have different pH and ion requirements for their optimal activity and/or release. Enzymic action following prolonged incubation in a balanced salt solution has been shown to result in epidermal-dermal separation. We have investigated the effects of simple salt solutions on intraepidermal cohesion *in vitro* and have defined conditions (20–50 mM phosphate buffer, pH 7·4 at 37°C) which rapidly induce blisters that are histologically and ultrastructurally indistinguishable from those in pemphigus vulgaris. Acantholysis is slowed at 4°C and prevented by acidic pH or certain protease inhibitors, but is unaffected by cycloheximide suggesting that endogeneous neutral proteolytic enzymes are involved.

The possibility that the acantholytic agent is plasmin is suggested by the finding of fibrinolytic activity in the phosphate buffer extract in the presence of plasminogen, and the prevention of acantholysis by aprotinin, which inhibits plasmin but not plasminogen activator (PA).

These results are consistent with the current view that the unusual pattern of acantholysis characteristic of pemphigus vulgaris results from the release of PA which then interacts with endogeneous plasminogen to produce plasmin. However, they further show that this release can occur as a result of a non-immunological stimulus, and that it can involve endogenous rather than newly synthesized PA. Such a mechanism may underly the production of pemphigus-like lesions as a consequence of drug treatments or burns.

Do plasminogen activators have a function in normal human epidermis?

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Plasminogen activators (PA) have been shown to have activity in cultured human epidermis (Hashimoto *et al.*, *J. Invest. Dermatol.* 1983, **81**, 424). Their action has also been implicated in the processes of acantholysis, wound healing and terminal differentiation. We have also been able to demonstrate PA in cultured human epidermal cells by autoradiography *in situ*; by radial diffusion in fibrin-agar plates after sonication and extraction of cultures with 2 M KSCN, and by separations of this extract by SDS–PAGE followed by zymograms of plasminogen-rich fibrin-agars. In agreement with the above authors we believe the PA present in cultured cells is of the urokinase type (mol. wt. 55K). In addition, conditioned medium from serum-free cultures showed PA in the 35–40K mol. wt. range. However, the autography produced by larger epidermal cell colonies *in situ* was typically distributed at the periphery and often around one or two cells. These may be migrating cells or contaminating fibroblasts. The latter have been shown to release PA (mol. wt. 68K) (Cederholm-Williams & Porter, *Br. J. Dermatol.* 1984, **110**, 423) but though our fibroblast cultures demonstrate PA in some cases we have not yet identified the molecular weight range. However, separation of normal adult human epidermis from biopsies by CaCl or trypsin and homogenization with 2 M KSCN and sonication shows no activity on zymograms or fibrin-agar plates. Further, cell suspensions from biopsies do not exhibit PA activity. Finally, horizontal cryostat sectioning of normal skin shows no activity at any layer of the epidermis compared with the blood vessels in the papillary dermis. Plasminogen activation may therefore be a phenomenon induced by disease (pemphigus) or uncontrolled growth (tissue culture, psoriasis, wound healing) and may have little function in normal differentiation.

Subclinical abnormalities of irritated skin

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Patients who itch but on whom no lesions are visible continue to puzzle clinicians. Patients with acute eczema of one site often develop eczema elsewhere after a relatively minor stimulus and may also develop multiple false patch test reactions (the 'angry back' syndrome). These observations suggest that there may be minor structural or functional abnormalities of the epidermis that are not clinically obvious but can give rise to symptoms and predispose to eczematous change. In this study we have attempted to characterize such abnormalities in both patients and experimental subjects. Application of dithranol in acetone in 0·1 and 0·5 of the minimal irritancy dose (MID) to the skin of volunteer subjects resulted in a 212% and 476% increase respectively in Laser–Doppler readings (indicating increased rate of blood flow) from the test sites after 24 h compared with control. Also measurement of transepidermal water loss with an evaporimeter at the dithranol-treated sites revealed an increased rate of loss at the 0·5 × MID site compared with control (166%). Similar observations were made at sites treated with UVR below the erythema threshold (0·5 × MED). Studies of the uninvolved skin of patients who have had eczematous diseases in the recent past demonstrated decreased mean corneocyte area and an increased tendency for the corneocytes to clump, and increased reactivity to a chemical trauma (minimal blistering time to 50% NH_4OH). These results indicate that normal appearing skin may have minor structural or functional abnormalities that could give rise to symptoms and could be regarded as a 'pre-eczematous' state.

Neurofibromatous dermal hypoplasia. A clinical, pharmacological and ultrastructural study

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We noted areas of depressed thin skin in two patients with neurofibromatosis (NF). A further twenty-five patients with NF were surveyed and one more example was found. The largest of these areas of neurofibromatous dermal hypoplasia (NDH) measured 9 × 5 cm and skinfold thickness was reduced below 50% of normal adjacent skin. The minimal trauma caused by the Harpenden calipers induced a triple response in the surrounding skin but not in the lesion.

Intradermal injections of the vasodilators histamine, bradykinin and prostaglandin E produced no reaction within the areas of NDH whilst noradrenaline produced attenuated vasoconstriction compared to the surrounding skin. The vessels within the NDH lesion thus appeared to have markedly diminished responses to vascular stimuli.

Light microscopy showed the dermis to be thinned and infiltrated with cells and stroma typical of NF. Electron microscopy showed cells with ovoid nuclei and long thin cytoplasmic processes that were tightly wrapped around blood vessels. These cells had a discontinuous basement membrane and pinocytotic vesicles, typical of perineurial cells.

We hypothesize that the lack of vascular reactivity in NDH is due to perineurial cell processes wrapping around dermal vessels acting as both a physical splint to prevent vasodilation and as a barrier to diffusion of pharmacological agents.

Investigation of lamina lucida blisters using fluorescein-labelled antibody and lectin probes

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We have attempted to characterize further the site of cleavage in a number of blistering conditions, which have been shown by conventional EM to separate within the lamina lucida (LL) of the epidermal basement membrane. Fresh unfixed tissue was obtained from natural lesions of patients with bullous pemphigoid and junctional epidermolysis bullosa, and from suction blisters in healthy volunteers and patients with recessive dystrophic epidermolysis bullosa (RDEB).

Four micrometric frozen sections were examined by indirect immunofluorescence using antibodies to bullous pemphigoid antigen (BPab), type IV collagen (IVab), laminin (LNab), a novel amnion-derived antigen (Aab), as previously described (Ortonne *et al.*, *J. Invest. Derm.* 1983, **80**, 380), and by direct microscopy using fluoresceinated *Bandeiraea simplicifolia* lectin I (BSL-I).

IVab localized to the blister floor in all cases, while BPA was demonstrated in the roofs of natural and suction blisters, confirming LL splits. LNab bound to floor and roof in all blisters except in bullous pemphigoid in which it was confined to the floor. Aab was found only on the floor in junctional epidermolysis bullosa and bullous pemphigoid, but on the floor and roof of normal suction blisters and of those from RDEB subjects. BSL-I was found in the floor of all blisters.

We believe these findings suggest the existence of different cleavage planes and question the possibility of antigenic subdomains, within the LL. Previously thought to bind with laminin in animals, BSL-I did not appear to co-distribute with laminin in this study.

The localization of circulating IgA antibodies in linear IgA disease, chronic bullous disease of childhood and childhood cicatricial pemphigoid using suction blisters as substrate

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The ultrastructural localization of IgA antigen in linear IgA disease of adults (LAD) is reported to be in the lamina lucida (LL) or below the basal lamina (Yaoita & Katz, *J. Invest. Dermatol.* 1977, **69**, 558). Suction blisters provide a consistent split through the LL and hence a means of localization of the reaction site of circulating anti-basement membrane zone (BMZ) antibodies.

Suction blisters were raised in normal volunteers, removed by punch biopsy and snap-frozen in CO_2/hexane bath. Five micron thick sections were used as a substrate for the indirect IMF techniques to determine whether the circulating IgA antibodies present in LAD, chronic bullous disease of childhood (CBDC) and childhood cicatricial pemphigoid (CCP) are directed against components of the LL or sub-basal lamina. The level of the split was confirmed using antilaminin.

Serum from two patients with LAD, eight with CBDC, three with CCP, five with BP and five with classical dermatitis herpetiformis (DH) were tested.

We found that the circulating IgA antibodies in LAD were bound to the roof of the blisters as well as the base. In CBDC they were chiefly at the base of the blister, and in CCP mainly at the roof of the blister. The circulating IgG antibodies in bullous pemphigoid (BP) sera were bound to the roof and the base of the blister, as previously described by Gammon *et al.* (*J. Invest. Dermatol.* 1984, **82**, 139). Sera from DH patients were negative.

These results demonstrate that the antigens in the various linear IgA dermatoses are distinct and localized to different sites. The LAD antigen is present in the lamina lucida and associated with the basal lamina. The CBDC antigen is closely associated with the basal lamina, that of CCP is in the lamina lucida.

Anti-amnion antibody specifies abnormality of the dermo-epidermal junction (DEJ) in junctional epidermolysis bullosa (JEB)

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Recent studies (Ortonne *et al.*, *J. Invest. Dermatol.* 1983, **80**, 380) have shown that an anti-amnion antibody (AAAb) binds to a lamina lucida component in normal skin and other epithelia and that the localization of the antigen is different from that of various recognized components of the basement membrane zone.

Using indirect immunofluorescence, we studied AAAb reactivity in skin from eight patients with different types of JEB. Three infants had lethal disease (LJEB), two children aged 2 and 6 years had severe indeterminate JEB and three adults had non-lethal disease (NLJEB). In addition, we examined the skin from two 19 week fetuses with LJEB. In lesional and non-lesional skin samples from the two fetuses and all patients with either LJEB or indeterminate JEB, the degree of AAAb fluorescence was markedly reduced compared with normal fetal and adult skin. In lesional skin, AAAb consistently localized to the blister base. In two patients with NLJEB the staining intensity was normal in blisters and non-lesional skin, whereas in the third patient with life-long severe disease, the staining was comparable to that found in the lethal and indeterminate groups. In recessive dystrophic epidermolysis bullosa (one patient) and in bullous pemphigoid (one patient) DEJ staining with AAAb was normal. Furthermore, the pattern and intensity of fluorescence with both bullous pemphigoid and laminin antibodies appeared normal in JEB.

These findings suggest that AAAb specifies an abnormality of the DEJ in JEB which may correlate with disease severity.

The fate of Langerhans cells in tissue culture

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The fate of Langerhans cells (LC) in epidermal cell cultures has been studied. Fresh normal human skin was obtained from mastectomy specimens, and epidermal cell suspensions prepared by overnight trypsinization at 4°C. The cells were then put up in tissue culture in DMEM medium with 10% fetal bovine serum at pH6, and plated on to plastic Petri dishes at a density of $0{\cdot}5 \times 10^6$ cells/cm^2. LC numbers were assessed by immunofluorescence labelling with monoclonal antibodies OKT4, OKT6 and OKIa, in the original cell suspension, and at days 1, 2, 3, 5, 6, 7, 9 and 10 in the supernatant and adherent monolayer of cells which were removed by warm trypsinization. Samples were also submitted for standard transmission electron microscopy. The results are the mean of four separate experiments. In the original suspensions, LC were 3–7% cell population. At day 1, the majority of LC were still in suspension in the supernatant, and < 1% were adherent. LC persisted in the adherent monolayer in small numbers until day 6/7 at which time no LC could be found in the growing cell population. EM studies showed progressive degenerative changes in the LC present, and a marked increase of Birbeck granules in some cells. No LC in mitosis were observed, and no LC were observed at EM after day 7.

These studies indicate that LC do not adhere well to plastic and are lost by day 7 from epidermal tissue cultures. This may indicate that the cells are terminally differentiated and are incapable of further growth, or that essential growth factors for LC are missing from our culture conditions. Loss of LC from epidermal cultures may have therapeutic implications in their use in wound coverage, as Ia bearing cells are no longer present to act as the focus for graft rejection.

Measurement of stratum corneum renewal time with dansyl chloride fluorescence using quantitative techniques

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Dansyl chloride has been widely used to assess the rate of stratum corneum turnover, by observing the disappearance of fluorescence after application of this agent to the skin. Measurement of the degree of fluorescence should give greater precision and make the technique easier to use. A previous study using a photographic densitometric technique has shown a turnover time can be predicted after 5 days at the site of fluorescence. This process is a time-consuming, two-step procedure, requiring specialized equipment. Attempts at scoring the degree of fluorescence by observation can distinguish different rates of stratum corneum (SC) loss but in our experience are subject to considerable observer error and are not sensitive.

A new simple technique has now been devised which eliminates these problems, using a specially constructed fluorescence comparator. Measurements made using this device are based on the observer matching up a standard fluorescence strip visually with the fluorescing test site by decreasing the fluorescence of the standard using an optical neutral density 'wedge' filter.

Measurements of fluorescence using this technique have been made on ten subjects, simultaneously incorporating the photographic technique. Similar results from both techniques were obtained. The new technique shows low inter- and intra-observer error (coefficients of variation 4·7 ± 0·9 and 5·0 ± 1·8 per cent). The comparator described was able to discriminate the differing stratum corneum renewal rates after varying degrees of mechanical abrasion and adhesive tape stripping. The technique was also able to derive renewal times for plantar stratum corneum at three sites (heel, instep and sole—16, 26 and 16 days respectively).

Use of the comparator for the dansyl chloride fluorescence techniques makes it a more rapid method of assessment and gives objectivity and sensitivity to the method.

Skin fibroblast culture using the collagen lattice

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Human skin fibroblasts cultured in a hydrated lattice of type I collagen appear to be a closer model of the living dermis than any hitherto available. Contraction of the disc-shaped lattices is a conspicuous activity of fibroblasts from normal skin (Bell *et al.*, *J. Invest. Dermatol.* 1983, **81**, 2s) but transformed cells and fibroblasts from patients with dystrophic recessive epidermolysis bullosa (EBDr) are reported to be less effective (Ehrlich *et al.*, *J. Invest. Dermatol.* 1983, **80**, 56). In exploratory studies we have compared the contraction caused by twenty-two different fibroblast lines from normal and abnormal skin and by several other cell lines. There was the expected strong correlation between the degree of concentration and the number of cells in the lattice ($r = -0{\cdot}745$; $P < 0{\cdot}001$) but only small differences between fibroblast lines.

Fibroblasts from four types of epidermolysis bullosa, including three EBDr lines, caused similar contractions to normal fibroblasts, but those from a patient with Werner's syndrome caused less contraction and NCTC 2544 epithelial cells almost none.

Griseofulvin added to the lattice mixture at 2–16 μg/ml produced a dose-dependent inhibition of contraction ($P < 0{\cdot}01$). Four corticosteroids at 10 μg/ml also inhibited contraction, the order of increasing effect being betamethasone valerate, hydrocortisone, clobetasone butyrate and clobetasol propionate. The glycosaminoglycan secreted by fibroblasts in lattices was predominantly hyaluronic acid, as in monolayer cultures, and the same cell density-dependent control of secretion was apparent. These findings are further evidence that the collagen lattice is a useful new source of information on the behaviour of cultured skin cells and on the interactions of connective tissue components.

Foot infection in coal miners

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It is 25 years since the classic study of dermatophyte foot infection in miners. A new survey has been conducted in two Nottinghamshire pits sampling 514 men. Seventy per cent of men have some degree of foot infection. About one-third of this is associated with dermatophytes and much of the remainder with Gram-negative bacilli. A number of features indicate that dermatophyte and bacillary infection should be considered separately. For example, feet that itch are most likely to be infected with dermatophytes; feet that get wet during the working day are more prone to bacillary infection; the prevalence of dermatophyte infection rose in proportion to years of service in the coal industry; bacillary infection was common throughout. The miner's contention that no doctor knows how to treat foot infection may be a reflection of the two separate entities.

The pathology of affected and uninvolved skin during treatment of acne with isotretinoin

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Isotretinoin is a highly effective treatment for the inflamed lesions of acne. Its mode of action is not fully established. Possible mechanisms include its action as a sebostatic or anti-inflammatory agent, or its effects on epidermal differentiation. We have studied the histology of affected skin (early papules) and skin from acne areas in patients receiving isotretinoin for treatment of nodulo-cystic acne.

In the first study, informed consent was obtained from fifteen patients receiving isotretinoin 0·5 mg/kg/day. Biopsies of small inflamed papules were taken before and during treatment and examined histologically. The amount of sebaceous gland tissue present, the overall degree of inflammation and the proportion of individual inflammatory cell types were assessed 'blind' on analogue scales. There was a statistically significant decrease in the amount of sebaceous gland tissue present during treatment compared with before treatment but no change in the amount or morphology of the inflammation at any time. This would suggest that isotretinoin is preventing the evolution of new acne lesions rather than acting as an anti-inflammatory agent. In previous work we have shown that stratum corneum (SC) released into the dermis produces an inflammatory process closely resembling that seen in acne. By reducing sebum secretion, isotretinoin may prevent the rise in pressure inside closed comedones and their subsequent rupture or leakage, thus preventing exposure of SC to the dermis.

Studies on the keratinization of the sebaceous ducts and more detailed investigation of sebaceous glands are at present under way in patients before and during treatment with isotretinoin 1 mg/kg/day, and results will be reported.

British Journal of Dermatology (1984) 111, 733–738.

Correspondence

Migration and mitosis in the epidermis

SIR, I am grateful to Potten's group (*British Journal of Dermatology* 1984, 111, 695) for pointing out an error for which I apologize. On page 635 and then on page 636 I wrote 'mouse epidermis'. It should have been 'guinea-pig epidermis'. My work was concerned with specifically analysing the data presented in Figs 1, 2 and 6 by Etoh, Taguchi & Tabachnick (1975) and, as Potten and his colleagues correctly state, Etoh *et al.* in their paper present data almost entirely from guinea-pig epidermis. The confusion between mouse and guinea-pig epidermis might have generated a certain amount of misunderstanding, but it seems to me that their Comment article indicates a more basic misunderstanding of the significance of my theoretical analysis (Mitrani, 1983).

The computer simulations reported by Mitrani (1983) ask, and in my opinion answer, a very specific question: Is it true that the experimental data presented by Etoh *et al.* (1975) cannot be explained on the basis of the passive migration hypothesis? I claim that the answer to this question is no. I have shown that the parameters that can explain the kinetics of normal guinea-pig epidermis can produce depopulation values comparable to those found by Etoh *et al.* (1975) after irradiation, when I assumed migration to be passive.

The strategy of the computer experiment was to feed into the model, figures that would closely represent those curves (Figs 1, 2 and 6, open circles) reported by Etoh *et al.* (1975) on normal guinea-pig epidermis. In the computer simulation I assumed a growth factor for the basal layer of 1·0 and a mitotic rate of 0·0044. Using the figures reported, the cycle time can be derived from the formulae presented earlier (Mitrani, 1982).

Every effort was made to use measurements derived from experimental data, yet strictly speaking, all that was required was to find any configuration which could kinetically describe normal guinea-pig epidermis. Thus, although it is evident that 'adoption of different assumptions will strongly influence the position and slope of the theoretical curves', once the model is set to a particular configuration and tested against the normal situation (i.e. against Figs 1, 2 and 6, open circles, Etoh *et al.*), one cannot change the parameters or adopt other configurations than those stated. Instead, one proceeds to 'experiment' with the computer model, in the same way as Etoh *et al.* did when they irradiated guinea-pig epidermis, by stopping mitosis in the simulated system. Under those conditions, without altering the parameters of the configuration, one then compares the resulting curves with the experimental data obtained after irradiation (i.e. Figs 1, 2 and 6, closed circles, Etoh *et al.*).

In my paper I reported a configuration which was experimented with, under two different assumptions: the first assumed that migration is an active process as suggested by Etoh *et al.* This assumption yielded depopulation values far higher than those observed experimentally once mitosis was suppressed. The second assumed that migration is a passive event and generated curves which are consistent with those obtained by Etoh *et al.* after suppression of mitosis. Since the configuration was created on the basis of the data generated by normal guinea-pig epidermis it comes as no surprise that 'neither model fitted the results for pig skin' presented by Potten's group above. The configuration, under the passive migration assumption, did fit quite nicely, however, the actual data from guinea-pig presented above by Potten's group (Fig. 1B).

I have not claimed that the solution presented is unique. It may be possible to find another configuration, under different assumptions, that may satisfy the requirements stated above.

I fully agree with Potten's group that normal mouse epidermis does not seem to be 'under pressure'. In fact this is in agreement with my theory which predicted that for mouse epidermis and for other epidermal systems of low turnover the number of basal cells produced per unit time is lower than the number of cells available to be extruded per unit time from the basal layer. A pressure situation is unlikely in such a system (Bullough & Mitrani, 1976, 1978; Mitrani, 1978, 1983). I have however shown that measurements that can generate kinetic curves comparable to those reported by Etoh *et al.* for normal guinea-pig epidermis can generate a situation in which a certain degree of population pressure can build up in the basal layer. I have further shown that such population pressure is *sufficient* to produce the depopulation values which were observed experimentally once mitosis was suppressed.

I believe that a theoretical analysis of the type presented is important for several reasons. In a restricted sense, it allows one to tackle the problem of active *versus* passive migration in ways other than having to ask which comes first, mitosis or migration (Potten & Allen, 1975). In a more general context, it presents a way to examine to what extent hypotheses, built on the basis of experimental data, are indeed consistent. The simplistic approach that theoretical models are unrestricted and therefore shapes of curves can be changed at will is probably the result of insufficient understanding of what a theoretical simulation entails, and can only delay the reduction of the degree of subjective interpretation of data derived from biological experiments.

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The prevalence of water-induced itching

SIR, Proposed criteria for the diagnosis of aquagenic pruritus (AP) were recently discussed (Steinman & Greaves, 1984). The condition presents with intense and often extremely distressing pruritis after contact with water that occurs regardless of the water temperature or salinity. There are no observable skin changes. Mental irritability may also be a prominent associated feature. As presently defined, AP is a rare condition. However, we have suspected that itching of the skin after water contact is a common phenomenon. We therefore carried out a simple questionnaire survey to determine the approximate prevalence of water-induced skin itching in a population of dermatology out-patients.

Questionnaires were given to consecutive patients attending general dermatology clinics at St John's Hospital for Diseases of the Skin, London, during the months January to April, 1984. The patients' age, sex and primary dermatological diagnosis were recorded in all cases. The dermatological diagnoses were broadly classified as pruritic or non-pruritic for the purpose of subsequent analysis. The patients were asked whether they had ever noticed redness, rash, itching, prickling or skin dryness after bathing or swimming. Additional questions referred to the duration and severity of the symptoms, their regularity, the effect of water temperature, and the presence or absence of emotional irritability concurrent with the skin itching. We did not attempt to ascribe an exact explanation for the water-induced skin itching in all of the patients. However, if patients' replies suggested that they may have true AP, they were sent a subsequent questionnaire to clarify and amplify their responses.

Completed replies were received from 363 patients (182 male and 181 female), ranging in age from 3 to 75 years. The essential findings are shown in Tables 1 and 2. One hundred and thirty-seven patients (37·7%) reported experiencing itching after contact of the skin with water. However, only 10 of these 137

TABLE 1.

Effect of water	Male	Female	Totals
Itch alone	7	3	10
Itch + *	69	58	127
No symptoms	106	120	226
Totals	182	181	363

* Itch with one or more of: redness, rash or dryness.

TABLE 2

	Dermatological condition		
Effect of water	Pruritic	Non-pruritic	Totals
Itch alone	5	5	10
Itch + *	95	32	127
No symptoms	100	126	226
Total	200	163	363

* Itch with one or more of: redness, rash or dryness.

mentioned itch as their only symptom after water contact. The other 127 patients experienced various additional concurrent symptoms (i.e. redness, rash, or dryness). This suggests, but does not prove, that their itching may have had a cause other than true AP. Possible explanations include exacerbation by water of a pre-existing pruritic dermatosis (such as eczema), or other conditions known to be associated with itching after contact with water. These conditions include aquagenic, cholinergic, cold, or heat urticaria; symptomatic dermographism, or polycythaemia rubra vera (Greaves *et al.*, 1981).

Further evaluation of the ten patients who reported itching as their only symptom after water contact suggested that none had AP. Five had a pruritic dermatosis which may have explained their itching after water. The other five, without a pruritic dermatosis, had one or more factors distinguishing their symptoms from those of AP. These included (1) the brief duration of pruritus (e.g. 5 min or less), (2) the pruritus was not noted consistently every time they bathed or swam, (3) water temperature significantly affected the severity of the pruritus, or (4) the itching was only mild in severity.

In evaluating the questionnaire responses, we assumed that those patients (totalling 127) reporting redness, rash, or dryness concurrent with itching after water contact could not have AP. They were therefore not evaluated further. It must be acknowledged, however, that some of these patients may have been referring to the above skin changes in areas anatomically distant from those experiencing pruritus. Thus, cases of true AP may have been overlooked in this group.

We have shown that itching of the skin after water contact is a common symptom among dermatology out-patients. However, we were unable to identify a single case of AP, as currently defined, in this survey of 363 patients. AP thus appears to be an uncommon condition among dermatology patients, but a reliable measure of the prevalence of AP in the general population cannot necessarily be inferred from this study of a population of skin disease sufferers. Whereas true AP appears to be rare, it is probable that there are patients who experience itching of the skin after water contact, but to a milder degree than that required for the diagnosis of AP.

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Treatment of acne with isotretinoin

Sir, In their letter entitled 'Remission rates in acne patients treated with various doses of 13-*cis* retinoic acid (isotretinoin) (*British Journal of Dermatology* 1984, **111**, 123–124), Drs Cunliffe and Jones quote their previous paper (Jones, Greenwood & Cunliffe, 1983) in which they predicted that doses lower than 0·05 mg/kg/bw should be sebo-suppressive and of benefit in patients with mild acne.

Whilst agreeing with this prediction the authors and the manufacturers now think that only severe acne should be treated, and not mild acne at the lower doses. The benefit/risk ratio would not be justified in view of the fact that a non-teratogenic dose in humans has not been established and the risk would be increased in view of the greater patient exposure.

Drs Jones and Cunliffe stress the importance of obtaining a maximal clearance of severe acne using a 1 mg/kg/bw daily dose, thus also lessening the risk of relapse and the need for further treatment.

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REFERENCE

Jones, D.H., Greenwood, R. & Cunliffe, W.J. (1983) The dose–response relationship of sebum suppression to 13–*cis*-retinoic acid therapy in severe acne. *British Journal of Dermatology*, **109**, 366.

The investigation of patients with genital warts

Sir, Patients with genital warts who are referred to dermatologists seldom receive a comprehensive examination and investigation of the lower genito-urinary (g.u.) tract, unless the physician elicits symptoms of g.u. disease. However, Kinghorn (1978), in a study of patients with genital warts attending a department of g.u. medicine, found evidence of an additional genital infection in approximately one-third of the men and two-thirds of the women. Patients attending a department of g.u. medicine are largely self-selected and may represent a high-risk group for sexually acquired diseases compared with those referred by their general practitioner to a dermatology department. We therefore studied the latter group of patients to determine the incidence of additional genital infections in this population.

A total of forty-seven patients with genital warts were referred to the dermatology department during a period of 1 year; twenty-five unselected patients from this group were seen by two of us (GMF and BNS); all twenty-five patients, regardless of the presence or absence of additional g.u. symptoms, were referred to the department of g.u. medicine for investigation.

Thirteen of the patients were male, the mean age in this group was 25 years (range 19–43 years); eleven were single or divorced; two were married; none admitted to homosexual relationships. Twelve patients were female with a mean age of 22 years (range 17–35 years); ten were single or divorced and two were married.

In the department of g.u. medicine, after a full clinical examination, appropriate specimens were taken of urethral secretions in men, and of urethral, cervical and high vaginal secretions in women, and these were examined by Gram stain and direct microscopy for evidence of non-specific genital infection, gonorrhoea, candidal and trichomonal infections. In addition, inoculation into appropriate culture media for verification of *Neisseria gonorrhoreae*, *Chlamydia trachomatis*, *Trichomonas vaginalis* and *Candida albicans* was made. Where appropriate, cultures were also made for Herpes virus hominis. In all cases, serology for syphillis (V.D.R.L. and T.P.H.A.) was performed. In addition, in women, cervical cytology was routinely examined and, where possible, in both sexes, contact tracing was initiated.

The results showed three of thirteen male patients and eight of twelve female patients had evidence of additional g.u. infections; the findings in these patients are displayed in Table 1.

This study, although small, suggests that patients attending a dermatologist with genital warts have a comparable incidence of additional genital infections to those presenting at a department of g.u. medicine.

TABLE 1. Positive findings in referred patients

Age	Marital status	Diagnosis	Diagnosis in sexual partner (contact tracing)
Males			
20	Single	NSGI(+)	NSGI(+), genital warts
22	Married	Candidal balanitis	NT
19	Single	NSGI(−)	NT
Females			
35	Divorced	Trichomonas vaginalis and Candidal vaginitis	Candidal balanitis and genital warts
18	Single	Cervical dysplasia, NSGI(+)	NSGI(+)
18	Single	Nil	Genital warts
22	Single	Candidal vaginitis	NT
19	Single	Cervical dysplasia (with wart virus) Candidal vaginitis	NT
27	Single	Gonorrhoea, NSGI(+) Trichomonas vaginalis vaginitis	NT
22	Single	Gardnerella vaginalis vaginosis	NT
22	Single	Trichomonas vaginalis and Candidal vaginitis	NT
26	Married	Gardnerella vaginalis vaginosis	NT

NSGI = non-specific genital infection

(+) Chlamydia isolated on non-irritated McCoy cell media.

(−) Chlamydia not isolated.

NT = not traced.

Without the facilities and expertise in the fields of diagnosis, treatment and contact tracing, many of the infections, which may be asymptomatic, will not be diagnosed; clearly this is not in the best interests of our patients or their partners. The coexistence of other genital infections may also make the eradication of the genital wart virus more difficult (C.Cooper & H.S.K.Singha, personal communication). Furthermore, there is increasing evidence (Singer, Walker & McCance, 1984), of an association between the genital wart virus and cervical dysplasia. In our series only two of the twelve female patients showed cervical dysplasia as detected by a routine cervical smear, but in a larger study Walker *et al.* (1983) found intra-epithelial cervical abnormalities on colposcopy in twenty-eight out of fifty women with warts on the external genitalia. Although a causative role for the wart virus in cervical neoplasia is not proven, this finding is most worrying, especially as the incidence of genital warts and cervical carcinoma has at least doubled in the past decade.

For these reasons, we would strongly recommend referral of all sexually active patients with warts on the genitalia to a department with expertise in the investigation and treatment of these problems.

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British Journal of Dermatology (1984) **111**, 739–740.

Book Reviews

Immunodermatology. Edited by D.M.MacDonald (1984) London: Butterworth. Pp. 291. Price £35.

This book is the proceedings of a symposium on immunodermatology held in London in September 1983. The proceedings are, as was the meeting, divided into four groups. Two are related to diseases (eczema and vasculitis), one is on labelled antibody studies, and the last is on immunocompetent cells: function and recognition. Division into these four sections seems at times somewhat arbitrary, and purists may argue about the inclusion of drug eruptions and actinic reticuloid under the heading of eczema.

Those who participate in medical and scientific meetings are divided as to the value of published proceedings of this type. The reviewer's personal preference is for an ESDR style booklet of summaries sent to each registrant 1 to 2 weeks before the meeting and no further publication. This current volume tends to reinforce that view in that the majority of the better papers given at the meeting have already appeared elsewhere, in the *British Journal of Dermatology*, *Journal of Investigative Dermatology*, *Journal of the American Academy of Dermatology*, etc. In general, the clarity and definition of the illustrations in the journals are rather better than that achieved in this publication. The quality of papers in the volume is very varied, with some being presented as excellent, well-referenced articles, and others as a very brief one-and-a-half page summary. There are one or two unfortunate examples of a lack of due care being taken by either author or editor where black-and-white pictures have references in the legends to blue and brown staining.

R.M.MacKie

Atlas of Dermatology with Differential Diagnoses. 2nd Edition. G.Rassner and G.Kahn (1983) Munich: Urban & Schwarzenberg, Distributed in U.K. by Pitman, London. Price £22.95.

To be successful, an atlas of dermatology must convey accurately the colour, shape, location and such surface characteristics as scaliness of the skin and its diseases. Also there should be sufficient pictures to show the variations that one disease may have at different sites (e.g. psoriasis) and acquire with chronicity (e.g. atopic dermatitis).

This is the second edition of an atlas first published in 1978, with a text translated from German. The colour photographs are generally excellent, but there are too few of them to convey the variability of disease, e.g. there is only one adult seborrhoeic dematitis. Some are misleading, e.g. the squamous carcinoma and the keratoacanthoma look indistinguishable, and the text to the illustration entitled lentigo malignant melanoma proceeds to say it is a superficial spreading melanoma. Notable omissions include carcinoma metastatic to the skin, the ash leaf macule and the hypopigmented phase of pityriasis versicolor.

The text is longer than necessary to support the pictures, yet inadequate as an elementary textbook of dermatology (of which there is no shortage). The translation provides some opaque passages such as 'external and internal damage lead to pathology of the skin and mucocutaneous junctions especially where various types of biological organization occur' and frankly misleading statements such as 'many melanomas develop from use of hormones (oestrogens) and hydantoins'. The classification regards psoriasis as congenital, atopic dermatitis as an allergic eczema and does not include malignant melanoma with other skin tumours. There are some factual errors: for example, we are told there are 500 million hairs on the body, and 65% of female hair is lanugo in type.

Thus although the picture quality is good, there are more useful atlases than this one.

C.T.C.Kennedy

Chromium: Metabolism and Toxicity. Edited by D.BURROWS (1983) Boca Raton, Florida: CRC Press. Pp. 172. Price $77.

Chromium dermatitis remains an occupational scourge, while chrome ulceration is by no means a thing of the past. More recently, lung cancer has been associated with chromate production and asthma with chromate usage. It is timely, therefore, that an excellent monograph on the effects of chromium on man should have appeared recently under the capable editorship of Dr Desmond Burrows. The dermatologist will find in it five chapters that cannot fail both to deepen his understanding of allergic contact dermatitis from chromium and to widen his knowledge of the other effects of chromium in man.

Professor Love contributes a useful introductory chapter on the general biology of chromium and the methods for its analysis. This chapter contains essential practical data for anyone about to embark on any form of research involving chromium allergy.

The carcinogenicity of chromium for the lung is the main subject of Dr Lesley Bidstrup's excellent review and the entire subject of Dr Sverre Langård's. Dr Bidstrup also reviews the other effects of chromium on the respiratory tract, including bronchospasm and asthma.

Dr Desmond Burrows himself reviews the dermatological aspects of chromium, while Dr Ladislav Polak's chapter explores the immunology behind these dermatological effects. Dr Polak convincingly argues that an important way forward in the future prevention of chromium dermatitis lies in animal experimentation, and he reviews the results of such studies to date clearly and in detail.

The editor has cleverly assembled this monograph from the contributions of excellent and experienced workers in the field, including his own considerable experience of chromium dermatitis. The index is helpful and the general presentation and binding of the book is of high quality. It would have a useful shelf-life in any dermatological library.

R.J.G.RYCROFT

Cutaneous Lymphomas, Pseudolymphomas and Related Disorders. By G.BURG AND O.BRAUN-FALCO (1983) Berlin: Springer-Verlag. Pp. 542. Price DM 360.

Without doubt, the appearance of a volume devoted to cutaneous lymphoma fulfils a need that had been rapidly expanding.

This book, written, compiled and edited by Professors Burg and Braun-Falco, with the help of several renowned collaborators, is superb in many respects, although the chosen format generally lacks a continuous logical flow of information and ideas.

The format referred to concentrates in each chapter on individual diseases, designated generally in accordance with a modified Kiel classification (which may not coincide with everybody's concept). This arrangement has some advantages, notably the uncluttered ease with which individual entities may be reviewed—an excellent reference text. Moreover, a complex subject is made to seem relatively simple.

Of outstanding merit are the illustrations, including eighty-two colour and 150 black-and-white plates, each of which almost without exception is a model of clarity. For this virtue alone the book is well worth its price. The text is always concise, but perhaps suffers by this, as the descriptions are sometimes a little scant and occasionally inclined to idiosyncrasy.

If one or two criticisms are emphasized, they are certainly overwhelmed by numerous merits. Pathology and dermatology departmental libraries should be in possession of a copy, and I am sure that individuals interested in this field will have already purchased one. I would not be without it.

D.M.MACDONALD

British Journal of Dermatology (1984) **111**, 741–742.

News and Notices

B.A.D. Summer Meeting 1985. Closing date for Abstracts

The closing date for submission of Abstracts for the above meeting has now been brought forward to Tuesday, 29 January 1985.

British Association of Dermatologists

The Dowling Travelling Fellowship 1985

It has been agreed by the Executive Committee that the Dowling Travelling Fellowship should be awarded again in 1985. Applications are invited for the above Fellowship which is tenable for 1 year (value £5000). It is intended to help support a meritorious applicant who wishes to undertake a period of training and research abroad. Applications should be sent to the Honorary Secretary, British Association of Dermatologists, 7 John Street, London WC1N 2ES. Closing date 28 January 1985.

B.A.D. Travelling Fellowships

Travelling Fellowships for 1984 were awarded to Drs Hifziye Reshad, M.E.Rowland Payne, T.S.Sonnex and N.P.J.Walker.

Applications are invited for these Fellowhips for 1985 which will be awarded for a period of not less than 3 weeks. It is anticipated that Fellows would visit various centres either in the U.S.A. and Canada or in Europe, and attend one of the national meetings in dermatology. A grant will be given towards expenses. Application forms may be obtained from the Honorary Secretary, British Association of Dermatologists, 7 John Street, London WC1N 2ES. Closing date 28 January 1985.

Whimster Prize

The Whimster Prize for 1984 has been awarded to Dr Stephen M.Breathnach.

Bristol Cup 1984

The Bristol Cup awarded for the best poster at the Annual Meeting was presented to Drs R.C.Holmes and M.M.Black for their poster entitled 'Polymorphic eruption of pregnancy—a diagnostic dilemma resolved'. The runners-up were Drs C.B.Archer, H-J.Rauch, M.H.Allen and D.M.MacDonald with their poster entitled 'Studies on patients with carcinoid tumours'.

The Wycombe Prize

The Wycombe Prize for 1984 has been awarded to Dr R.Howell.

Applications are invited for this Prize which is offered again in 1985 for an outstanding contribution to dermatology published from a district general hospital or non-teaching centre in the United Kingdom. For regulations please contact the Honorary Secretary, British Association of Dermatologists, 7 John Street, London WC1N 2ES. The closing date will be 28 January 1985.

Stiefel Travelling Grants

We are pleased to announce that through the auspices of Steifel Laboratories (U.K.) Ltd., Travelling Grants for 1984 have been awarded to Dr Elizabeth Wong, Dr R.StC. Barnetson, Dr H.J.Dodd, Dr A.G.Messenger and Dr M.J.Tidman.

Applications are once more requested, and application forms and regulations can be obtained from the Honorary Secretary, British Association of Dermatologists, 7 John Street, London WC1N 2ES.

The Bristol-Myers Awards

The Bristol-Myers Awards for 1984 are as follows:

Research Travelling Scholarship. Dr Clifford M.Lawrence (Newcastle).

Two Travelling Fellowships for Consultants. Dr R.J.Pye (Cambridge), Dr E.H.Wyatt (Hull).

Two awards for the best oral presentations at the Annual B.A.D. Meeting. Dr C.P.Fitzsimmons, Professor Rona M.MacKie, Dr P.D.Wilson—'A study of the total number and distribution of melanocytic naevi in a British population'. Dr C.M.E.Rowland Payne, Dr N.A.Byrom, Dr J.Dixey, Dr C.Farthing, Dr J.I.Harper, Dr A.Lawrence, Dr P.Samarasinghe, Dr J.K.Oates, Dr R.C.D.Staughton—'Herpes zoster, homosexuality and atopy'.

Award for a highly meritorious paper in the B.J.D. Dr S.Forster, Dr E.Ilderton, Dr R.Summerley and Dr H.J.Yardley (1983) The level of phospholipase A_2 activity is raised in the uninvolved epidermis of psoriasis. *British Journal of Dematology*, **108**, 103–105.

Two Clinical Photographic Equipment Packages. Dr Dorothy France (Crawley Hospital). Dr Dafydd Roberts (Singleton Hospital, Swansea).

Applications for Bristol-Myers awards for 1985 are invited for:

(1) A research travelling scholarship open to Senior Registrars or Consultants to be used by a candidate who is travelling abroad for a specified item of research or training. It is envisaged that the scholarship will be for a period of about 3 months. Value £3000.

(2) Two travelling fellowships for Consultants. Value £750 each.

(3) A clinical photographic equipment package (two or three per year) to non-teaching units. Applicants are requested to give sufficient detail to help the selection committee to come to a fair decision. Applications should be sent to the Honorary Secretary, British Association of Dermatologists, 7 John Street, London WC1N 2ES by 28 January 1985.

Advances in Wound Management, Cardiff, March 1985

A conference will be held at the University of Wales, Institute of Science and Technology, Cardiff, on the subject of wound management, and this will take place on 20 and 21 March 1985. Further details are available from the Surgical Dressings Research Unit, Welsh School of Pharmacy, U.W.I.S.T., Edward VII Avenue, Cardiff.

Workshop on Dermatological Surgery, Newcastle upon Tyne, March 1985

A second Workshop on Dermatological Surgery will be held at Newcastle upon Tyne on 28 and 29 March 1985. The emphasis will be on basic surgical techniques with practical work on pigs' feet, but there will be lectures and discussion on related topics including cryotherapy and laser surgery. Details of the programme and accommodation may be obtained from Dr M.G.C.Dahl, Secretary, British Dermatological Surgery Group, Department of Dermatology, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP, U.K.

Fellowship of the Royal College of Physicians (London)

Dr R.S.Chapman has recently been elected F.R.C.P. (London).

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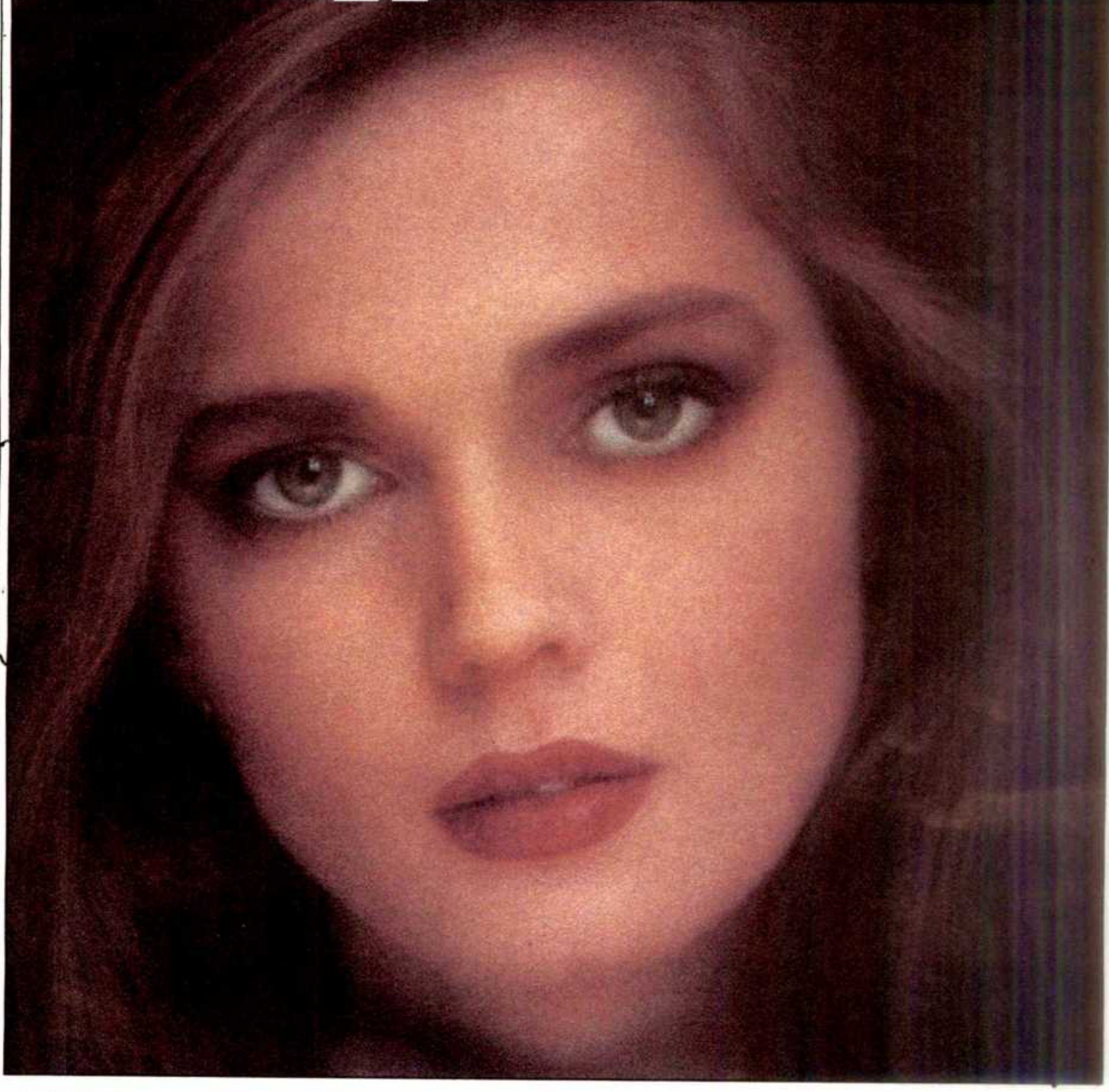

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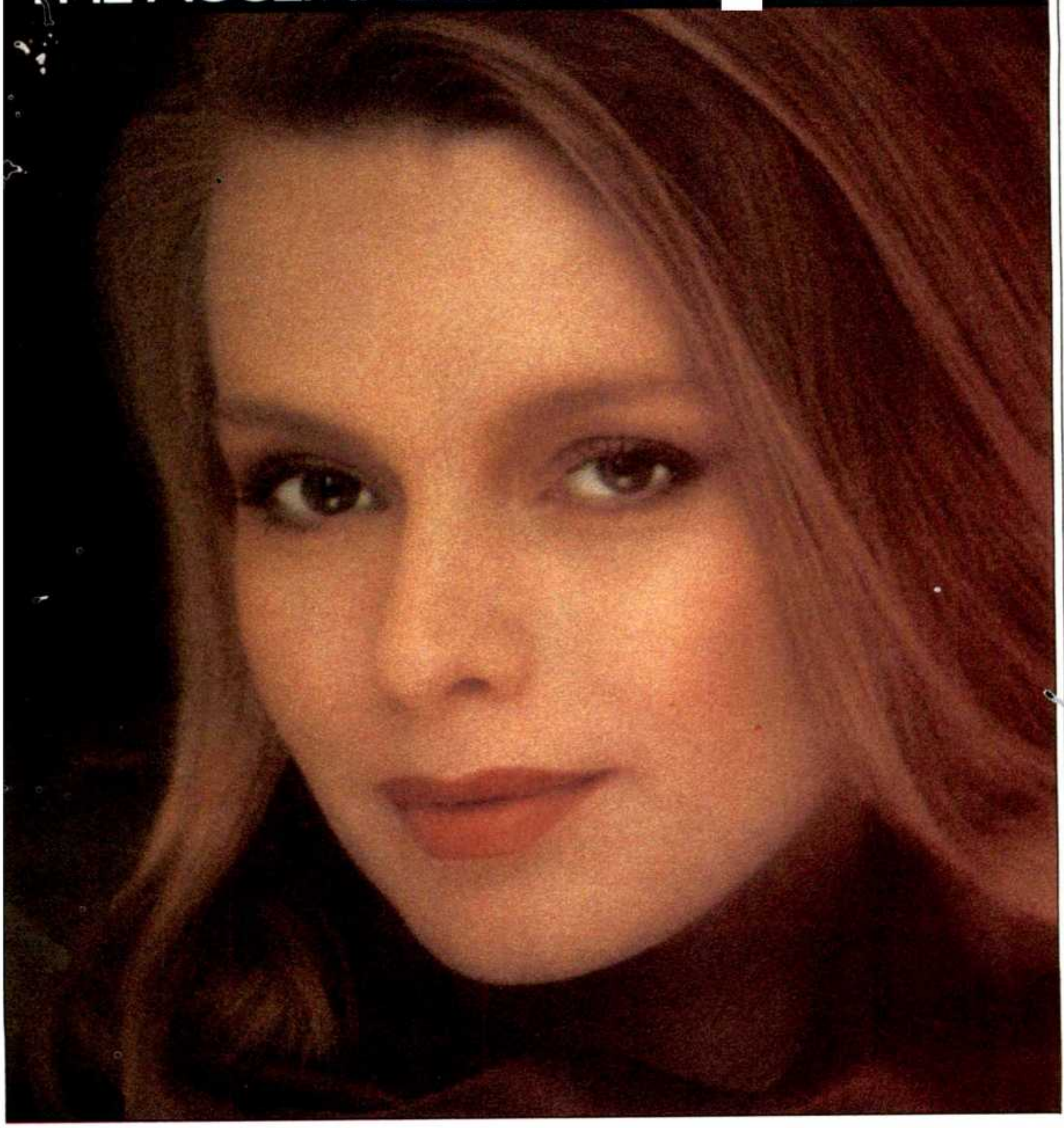

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3}g)	mg	second(s)	s	gravitational acceleration	***g***
microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hours(s)	h	milliequivalent	mmol		

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